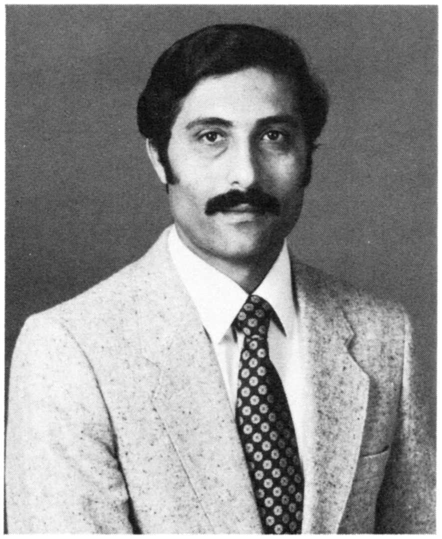
# Role of Calcium and Calmodulin in Plant Growth and Development

**B.W. Poovaiah**
*Department of Horticulture and Landscape Architecture,*
*Washington State University, Pullman, WA 99164-6414*

*Additional index words.* protein phosphorylation, signal transduction, cell function, physiological disorders

***Abstract.*** **The role of Ca as an intracellular messenger is beginning to be unraveled as a result of recent research on calmodulin, a ubiquitous protein that binds Ca ions and regulates various biochemical processes in plants. Experimental evidence suggests that certain cell functions in plants are regulated, in part, by Ca and calmodulin. Changes in cell wall rigidity, membrane permeability, and enzyme activation are known to influence various aspects of cell physiology and have a significant influence on the growth and development of plants. Deficiency of Ca is known to induce physiological disorders in fruit and vegetables. We hope that this article will stimulate further studies and provide new insights into how these problems may be controlled. The role of Ca ions in signal transduction and cell function is beginning to be understood at the molecular level, and we have embarked on a new phase of the old subject of mineral nutrition, especially as it applies to Ca and plant growth and development.**

It is well known that Ca plays an important role in plant growth and development. Physiological studies have shown that the soil solution must contain 1 to 5 mM Ca or growth would decline, with necrosis of the apical meristems (22). Calcium is essential for growth because both cell division and cell elongation require it (7, 35, 38, 48, 49, 54, 57). Calcium ions also are known to play an important role in the intricate network of interactions which controls the growth and development of plants (22, 32). Classical documentation of this fact has been demonstrated in physiological disorders caused by inadequate Ca (4, 13, 17, 19, 26, 60, 61, 62). Recent investigations have shown that there are changes in the intra- or intercellular distribution of Ca in response to various stimuli such as light and gravity (8, 38, 57, 69) and also suggest that changes in Ca distribution occur soon after the stimulus is perceived and before any macroscopic response is detected (57).


B.W. Poovaiah

One of the unique properties of Ca is that there is little or no redistribution following accumulation (22, 25). Many experiments provide the evidence that plants must be supplied continuously with Ca since the internal Ca is not redistributed to zones of new growth. For example, root tips require a continuous supply of Ca for their meristematic activity. A temporary cessation in the Ca supply can disrupt cell division and may cause death of roots.

For plants to function and adapt efficiently to the changing environment, cells must communicate with one another. All higher plants have built-in regulatory mechanisms which can be controlled in response to external and internal stimuli, such as gravity, light, and hormones. It is becoming increasingly evident that Ca ions are important intracellular messengers in plants. Since the discovery of calmodulin in mammalian systems (see 9, 10, 11 for reviews.), it is now clear that the Ca messages often are relayed by calmodulin, a ubiquitous Ca-binding protein. Information gathered from animal systems indicates that hormonal and neural stimuli are the 2 most important means by which individual cells respond to the signals from the rest of the organism. The signal received on the surface of the cell is communicated to the internal metabolic machinery of the cell by the second messengers, such as cyclic AMP (cAMP) and Ca (55, 56). Cyclic AMP is present in plants, but, in all probability, it is not a 2nd messenger (6). Therefore, there is increasing interest in defining the possible role of Ca as a 2nd messenger in plants (37, 38). Plant physiologists are trying to establish a similar role for Ca, and increasing evidence suggests that Ca fulfills all the criteria as a 2nd messenger in plants.

## Importance of Ca

Calcium has desirable effects on plant growth and development (31, 32, 48, 49, 50, 58). Calcium treatment delays ripening, senescence (45, 46, 64), and abscission (47, 52, 53) improves fruit and vegetable quality (3, 4, 20, 27, 36), alters geotropic response (29), α-amylase secretion (28), photosynthesis (5), and other processes such as cell division (7), cytoplasmic streaming (68) and cell enlargement (see 33 for review). Many physiological disorders of storage organs, such as bitter pit in apples (17, 51, 61, 62), blossom end rot in tomatoes, tip burn in lettuce, and hollow heart in potatoes are related to the Ca content of the tissue (3, 4, 14). Increasing tissue Ca levels usually diminish the occurrence of these disorders. Calcium is essential to maintain structural integrity of membranes and cell walls (33, 43, 44, 50).

---

Received for publication 25 Jan. 1984. Scientific Paper No. 6950, College of Agriculture and Home Economics, Washington State Univ., Pullman, Wash.; Project No. 0321. Supported in part by National Science Foundation Grant PCM-8208408 and Washington State Tree Fruit Research Commission. I thank K. Veluthambi, G. Paliyath, Joung Rhee, Yosef Mizrahi, K.G. Raghothama, Greg Glenn, and Mrs. V. Balamani for their valuable help. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1] Professor, Dept. of Horticulture and Landscape Architecture.

Under conditions of Ca deficiency, membranes become leaky, cellular compartmentation is lost (35), and Ca cross-linkage with the pectin in the middle lamella is affected. The importance of Ca in cell to cell adhesion is well recognized. The cementing effect is due primarily to the Ca pectate of the middle lamella laid down during cytokinesis (22). As cells grow, the area of surface contact between them increases greatly, and the need for constant supply of Ca becomes critical.

Aluminum toxicity in plants is of global interest since Al is the most abundant metal in the earth's crust. In acidic soils, Al becomes active, moving into plant roots. It is estimated that 40% of the arable soils of the world is acidic, and plants therefore are subject to Al toxicity (23). In the southeastern part of the United States, peach tree short life is related to Al-Ca availability or displacement. At low pH, Al ions interfere with Ca entry and/or uptake which is considered one of the several reasons for peach tree short life. Recently Siegel and Haug (63) have put forward a hypothesis indicating a complex formation between calmodulin and Al ions may be a key factor in Al toxicity. The toxic effects exerted by Al are known to interfere with Ca metabolism. Since calmodulin has been recognized as a multifunctional, Ca-dependent, regulatory protein for a variety of cellular responses (9, 10, 11), Al induced changes of calmodulin structure and function would therefore have severe repercussions on a multitude of cellular functions (see 23 for review).

In most plants, the bulk of the Ca is found in the apoplastic compartment, primarily complexed with cell wall moieties and the plasma membrane, in vacuoles and in other cytoplasmic organelles, such as mitochondria and endoplasmic reticulum where it is sequestered as salts of phosphate and various organic acids (12). The surface of the living cell can accommodate a rather wide variation in the composition and pH of the apoplastic solution only when there is adequate Ca in the free space. Concentrations of 1 to 5 mM Ca are required to protect the plasma membrane from the deleterious effects of low pH, salinity, toxic ions, and nutrient imbalance (22). Without such protection, the plasma membrane fails to discriminate between ions, the proton pump becomes disfunctional, and senescence is accelerated (43, 45, 46).

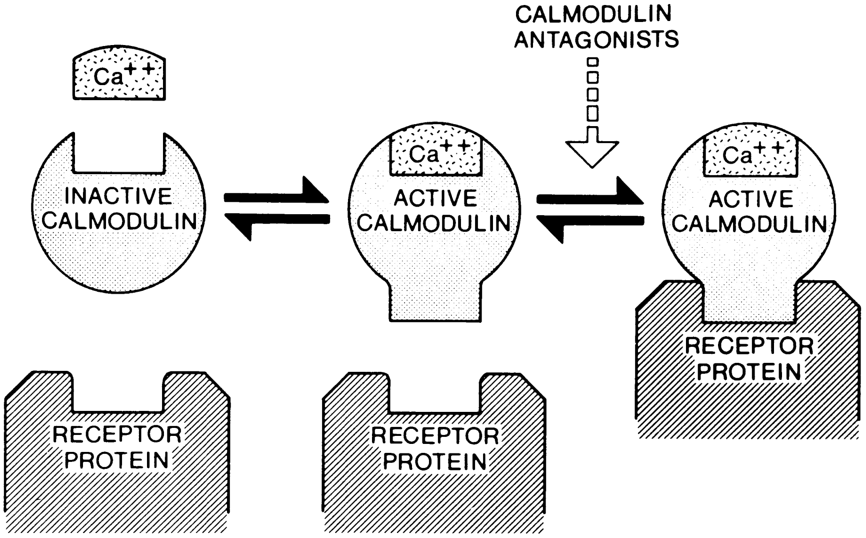


Fig. 1. Diagram illustrating the mechanism by which camlodulin mediates the biological action of Ca ions in plants. The first step involves the bindng of 4 Ca ions with the calmodulin molecule, thereby activating it. The activated calmodulin binds to the receptor protein (enzyme) and this leads to the active calmodulin-Ca-enzyme complex and the response is induced. If calmodulin antagonists are present (top arrow), the antagonists bind to the Ca-calmodulin complex, thus blocking the calmodulin response.

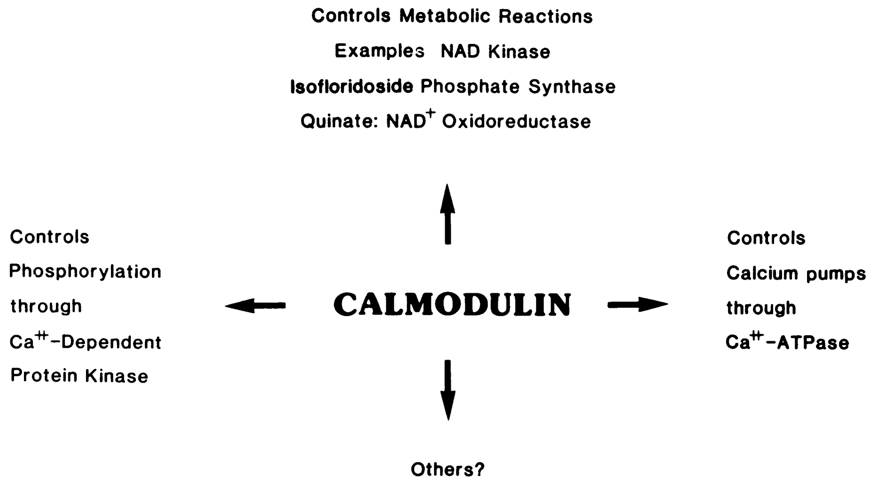


Fig. 2. Control of cellular functions by calmodulin. NAD-kinase synthesizes NADP from the cofactor NAD. Calcium-ATPase is the molecular pump that transports Ca ions from the cytosol to the "calcium stores". Protein kinases phosphorylate various proteins in a calcium- and calmodulin-dependent manner and it is emerging as the major, general mechanism by which calcium and calmodulin regulate biochemical events inside the cell in response to hormones and external stimuli. Other enzymes such as isofloridoside phosphate synthase and quinate: NAD$^+$ oxidoreductase are also known to be activated by calmodulin.

## Cytosolic Ca and its roles

In plants, as in animals, the level of free Ca in the cytosol is relatively low, in the range of $10^{-8}$ to $10^{-6}$M (9, 10, 11, 22). In contrast, vacuoles and other organelles are known to accumulate large quantities of Ca. Vacuolar Ca is separated from cellular metabolism as chelates and precipitates of organic acids such a oxalate and phosphate (22). Mitochondria have been found to contain as much as 700 nmole Ca/mg mitochondrial protein (16). Thus, an important point is that the free metabolically available Ca in the cytosol is in micromolar concentrations or even less. The low cytosolic Ca concentration is maintained by active Ca transport out of the cytoplasm by $Ca^{++}$-transporting ATPases on plasma membrane (39), mitochondria (70), and endoplasmic reticulum (37). Recent investigations by Dieter and Marme (16) provide convincing evidence that Ca-transporting ATPase activities of microsomal plant membranes are controlled by the regulator protein, calmodulin (CaM), in a $Ca^{++}$-dependent manner (15, 37).

## Plant calmodulin and its properties

The Ca-binding protein, calmodulin, seems to exist in all eucaryotic organisms. In 1977, Muto and Miyachi (39) discovered that there was a protein activator of NAD kinase present in plant extracts. Subsequent studies by Anderson and Cormier (1) confirmed that the activator protein from mammalian sources could regulate the NAD kinase activity of peas, thus demonstrating unequivocally that the protein activator of plant NAD kinase was, indeed, calmodulin (2). Calmodulin has been isolated and characterized from a large variety of animal and plant tissues, ranging from protozoans to mammals and lower to higher plants. Comparison of the physical and biochemical properties, including amino acid sequence of some calmodulins isolated from various sources, shows that calmodulin has been well conserved through the phylogenic scale.

Marme and Dieter (38) have provided evidence that the biochemical, biophysical, and biological properties of plant calmodulin are very similar to those of animal calmodulin. The molecular weight of zucchini calmodulin estimated by SDS-polyacrylamide gel electrophoresis is around 14,500 in the presence of Ca$^{++}$ as compared to 16,000 for bovine brain calmodulin (38). An interesting feature of calmodulin is its thermal stability and acidic nature. Boiling destroys most proteins, but calmodulin retains its activity even after boiling at 95° to 100°C for 3 min. Another striking characteristic of calmodulin is its lack of tissue or species specificity. Electrophoretic mobility and biological cross-reactivity of bovine brain, apple fruit, and corn coleoptlile calmodulins are quite similar. For example, an antibody directed against calmodulin from bovine brain recognizes calmodulin from plants (54, 58). In addition, all plant calmodulins tested in our laboratory stimulated calmolulin-dependent phosphodiesterase from mammalian sources (41, 52, 54). Recently, the structural properties of spinach (34, 71) and barley (59) calmodulins have been characterized. The amino acid composition of plant calmodulins indicate some minor differences in tyrosine, threonine, leucine, arginine, and lysine content compared to bovine calmodulin. The similarities suggest a fundamental role for calmodulin in mediating Ca$^{++}$-dependent processes in the cell (for reviews, see 9, 38, 57). The amount of calmodulin in plant tissues was determined by measuring the intensity of the protein band on SDS-polyacrylamide gels using bovine brain calmodulin as a standard (38). The yield of calmodulin was found to be about 10 mg per kg of plant tissue. This yield corresponds to about $10^{-6}$ to $10^{-5}$ M in the plant cell, with the assumption that the cytoplasmic space is about one-tenth of the volume of the vacuolated cell.

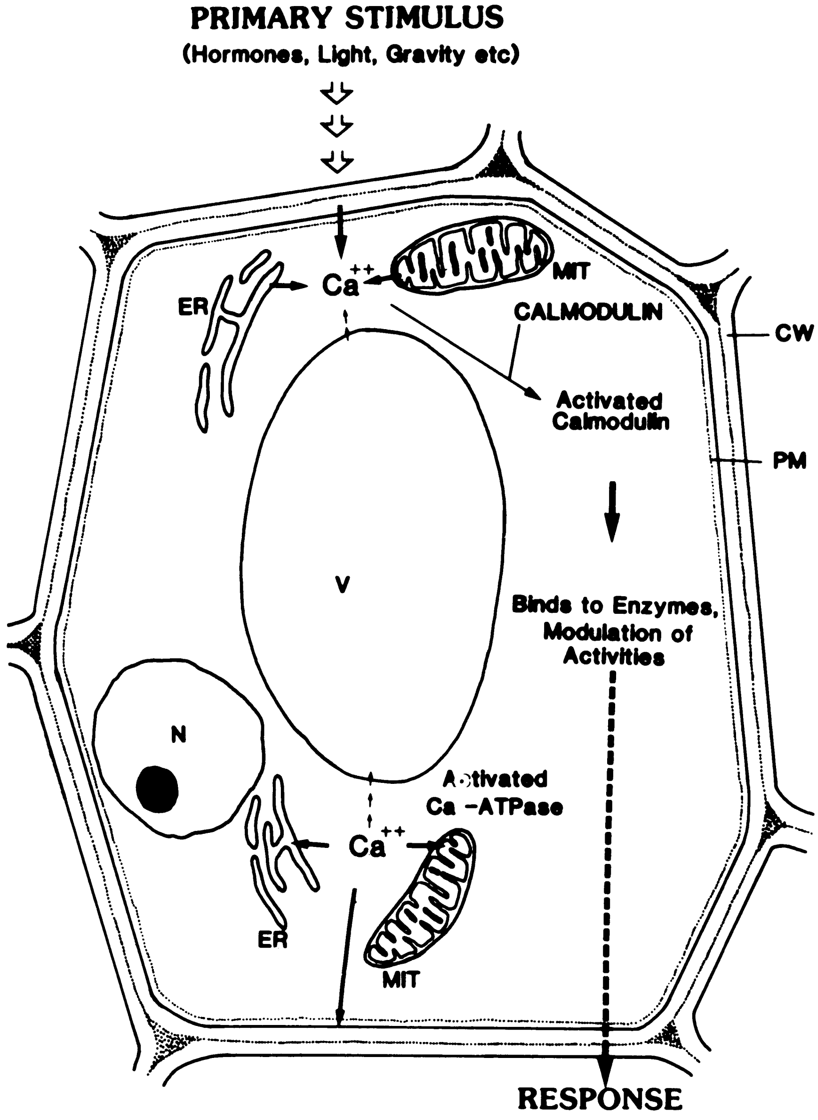


Fig. 3. Diagram illustrating the effect of primary stimuli such as hormones, light and gravity on cytoplasmic free calcium concentration and calmodulin-mediated changes in a plant cell. MIT: mitochondria, ER: endoplasmic reticulum, V: vacuole, N: nucleus, PM: plasma membrane, and CW: cell wall.

## Molecular mechanism of calmodulin action

Calmodulin is a regulatory protein found free in the cytoplasm and/or associated with membranes and organelles. A high level of Ca in the cytoplasm is injurious to the cell function. The steady state differential between the free space and the intracellular levels of Ca is maintained by the cell membrane, which is highly impermeable to the Ca ion. Ca-ATPase in the plasma membrane is involved in transporting excess Ca from the cell (21, 37), or Ca is taken up by intracellular organelles. When the cytoplasmic Ca level rises above $10^{-6}$ M, Ca ions bind to calmodulin, thus activating it. The role of calmodulin in mediating the biological action of Ca ions is shown diagramatically in Fig. 1. The first step in the sequence involves the interaction of Ca with calmodulin. The binding of 4 Ca ions to one calmodulin molecule causes conformational changes in the calmodulin, thereby activating it. Only the activated calmodulin (Ca-calmodulin complex) is able to recognize the receptor protein (enzyme) and bind to it. This binding leads to the active calmodulin-calcium-enzyme complex and, subsequently, the response. Enzymes such as Ca-ATPase (37), NAD kinase (1, 2), isofluoridoside phosphate synthase (29), and quinate: NAD$^{+}$ oxidoreductase (37) are activated in this manner (Fig. 2). The Ca-calmodulin complex also may bind to natural or synthetic calmodulin antagonists, resulting in a calmodulin-Ca-antagonist complex. This complex cannot activate calmodulin sensitive forms of enzymes, thus blocking the calmodulin-induced response (41, 42).

## Ca as a 2nd messenger in plants

External and internal stimuli, such as light, gravity, and hormones are known to alter the cytoplasmic free Ca concentration by controlling the Ca transport mechanisms. Although the concept of Ca as a 2nd messenger in plants is new, recent evidence supports such a role for Ca. This concept as it might apply to plants is illustrated in Fig. 3. As a cell perceives the message, Ca is released from "Ca stores" such as the apoplast region, mitochondria and endoplasmic reticulum into the cytosol. The increase in cytosol Ca concentration activates calmodulin, which, in turn, activates a number of enzymes (Fig. 2) leading to the response. Activated calmodulin also activates a Ca-ATPase which pumps Ca back into Ca stores. This will bring the cytosol Ca concentration back to the submicromolar range, thus gradually diminishing the calmodulin-controlled activation of various enzymes. The activation of several enzymes, such as phosphodiesterase, is dependent on calmodulin (Fig. 4) and Ca (38) concentrations.

## Posttranslational modification of proteins by Ca and calmodulin-regulated protein phosphorylation

It is a general biochemical contention that the changes associated with plant growth and development involve changes in the pattern of enzyme activities. As is now becoming clear, the posttranslational modification of enzymes may represent a key regulatory step in the molecular mechanism by which the majority of animal hormones operate. Enzymes can be modified in a number of ways, such as methylation, acetylation, thiolation, ribosylation, adenylation, and phosphorylation (65). In recent years, major interest has been directed towards protein phosphorylation (24, 30, 42, 66, 67, 68). The role of protein kinases in phosphorylation and phosphatases in dephosphorylation in plants has recently been demonstrated (67). Reversible protein phosphorylation offers a unique advantage in cellular regulation, since an enzyme activated upon phosphorylation by a protein kinase can be inactivated by dephosphorylation by a protein phosphatase. It is becoming clear that protein kinases play a major role in Ca-and calmodulin-regulated signal transduction in plants. Examples of Ca dependent protein phosphorylation in corn coleoptiles and tomato and apple fruit are shown in Fig. 5. Phosphorylation of several soluble polypeptides and membrane polypeptides was promoted by adding Ca. Calcium-promoted phosphorylation of some of the soluble and membrane polypeptides increased further in the presence of calmodulin.

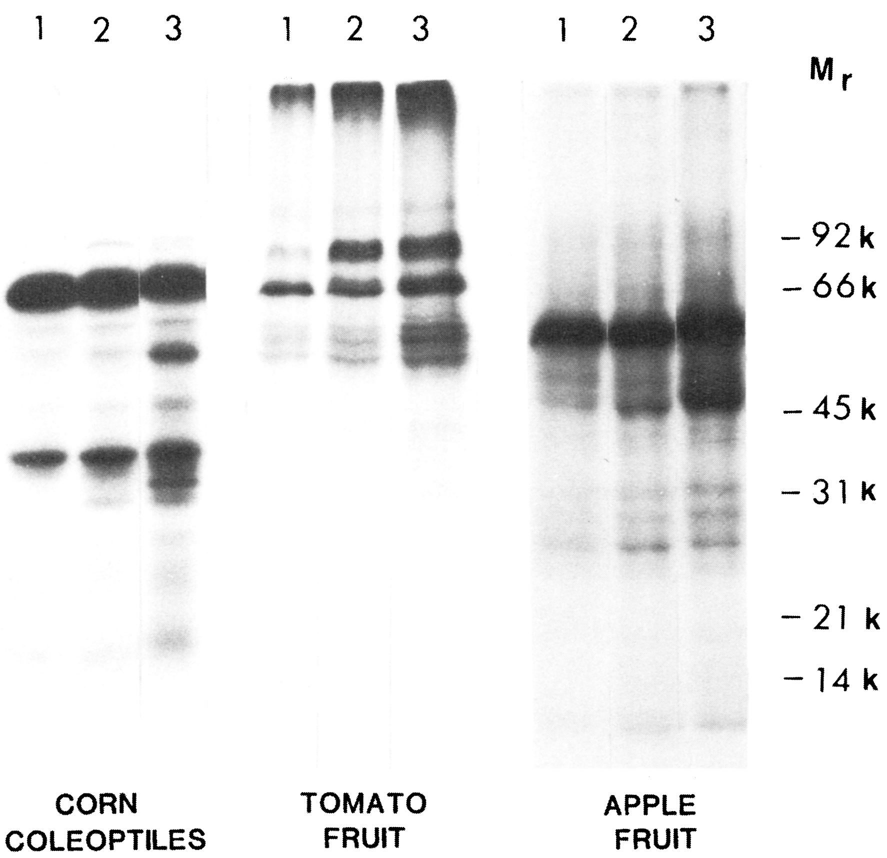


Fig. 5. Autoradiographs showing the effect of Ca and calmodulin on the phosphorylation of soluble polypeptides from corn coleoptiles and membrane polypeptides from tomato and apple fruits. Protein phosphorylation was carried out as described earlier by Veluthambi and Poovaiah (64, 65) using $[\gamma\text{-}^{32}P]ATP$. In vitro phosphorylations were performed in the absence of Ca (1), in the presence of 1 mM $CaCl_2$ (2) or in the presence of 1 mM $CaCl_2$ + 1.6 M calmodulin (3). The molecular weights of representative phosphorylated polypeptides are indicated.

Calmodulin antagonists, such as chlorpromazine, trifluoperazine, fluphenazine, and a naphthalenesulfonamide derivative (W-7), inhibit the auxin-induced cell elongation at micromolar range (54). Poovaiah and Leopold (46) have demonstrated that the senescence of leaves can be deferred by Ca application. Recently, we observed that the pretreatment of leaf disks with Ca chelators, such as ethyleneglycol-bis-(β-aminoethyl ether) N,N', tetracetic acid (EGTA), negated the cytokinin effect on senescence. The cytokinin effect lost due to EGTA pretreatment was recovered by transferring the leaf disks to a medium containing Ca and cytokinin (Rhee and Poovaiah, unpublished data). These results suggest an interaction between a plant hormone and Ca (32, 48) and a possible role for calmodulin in hormone action in plants (18, 54). Chlorpromazine and other calmodulin antagonists decreased Ca-promoted phyosphorylation (66, 67), suggesting that phosphorylation is modulated by calmodulin. The addition of Ca to the incubation medium promoted phosphorylation in a concentration dependent manner, and sodium molybdate, a known inhibitor of phosphatases which are involved in dephosphorylation, increased net phosphorylation (67).

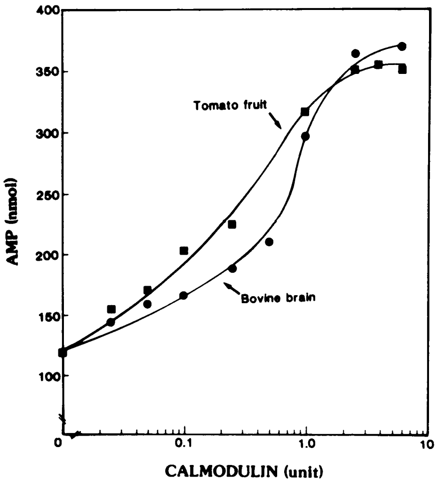


Fig. 4. Dose-response curve of the activation of calmodulin-deficient bovine brain cAMP-phosphodiesterase by calmodulin from tomato fruit and bovine brain.

In another series of experiments while assaying calmodulin activity in senesced apple extracts using its property of promoting the activity of phosphodiesterase from bovine heart, a heat stable, dialyzable low molecular weight component which inhibited calmodulin activity was detected (41, 42). The inhibition of calmodulin activity was reduced in extracts from apples where senescent breakdown was delayed by Ca treatment. These observations suggest a major role for calmodulin in plant growth and development and further suggest that calmodulin inhibitors are naturally present in some species and may play a role in calmodulin regulation.

Though various roles of Ca in cell function have been studied extensively in the past 20 years, the mechanism of Ca action is just beginning to be unraveled at the molecular level. There is no doubt that Ca and its binding protein, calmodulin, play a pivotal role in plant growth and development. A detailed understanding of the biochemical processes mediated by Ca and calmodulin would help in solving many of the Ca-related physiological problems in plants.

## Literature Cited

1. Anderson, J.M. and M.J. Cormier. 1978. Calcium-dependent regulation of NAD kinase in higher plants. Biochem. Biophys. Res. Commun. 84:595–602.
2. Anderson, J.M., H. Charbonneau, H.P. Jones, R.O. McCann, and M.J.Cormier. 1980. Characterization of plant nicotinamide Adenine Dinucleotide kinase activator protein and its identification as calmodulin. Biochemistry 19:3113–3120.
3. Arteca, R.N., B.W. Poovaiah, and L.K. Hiller. 1980. Electron microprobe and neutron activation analysis for the determination of elemental distribution in hollow heart potato tubers. Amer. Potato J. 57:241–247.
4. Bangerth, F. 1979. Calcium-regulated physiological disorders of plants. Ann. Rev. Phytopathol. 17:97–122.

5. Brand, J.J. and D.W. Becker. 1984. Evidence for direct roles of calcium in photosynthesis. J. Bioenergetics and Biomembranes 16:239–248.
6. Brown, E.G. and R.P. Newton. 1981. Cyclic AMP and higher plants. Phytochem. 20:2453–2463.
7. Burstrom, H.G. 1968. Calcium and plant growth. Biol. Rev. 43:287–316.
8. Chandra, S., J.F. Chabot, G.H. Morrison, and A.C. Leopold. 1982. Localization of calcium in amyloplasts of root-cap cells using ion microscopy. Science 216:1221–1223.
9. Cheung, W.Y. 1980a. Calmodulin plays a pivotal role in cellular regulation. Science 207:19–27.
10. Cheung, W.Y. (ed). 1980b. Calcium and cell function, vol. I, p. 395 Academic Press, New York.
11. Cheung, W.Y. (ed). 1982. Calcium and cell function, vol. II, p. 429, Academic Press, New York.
12. Clarkson, D.T. and J.B. Hanson. 1980. The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31:239–298.
13. Cocucci, M., S. Cocucci, I.L. Mignani, and C.P. Treccani. 1983. Possible relationship between bitter pit and membrane transport. Acta Hort. 138:43–51.
14. Collier, G.F. and T.W. Tibbitts. 1982. Tipburn of lettuce. Hort. Rev. 4:49–65.
15. Dieter, P. 1984. Calmodulin and calmodulin-mediated processes in plants. Plant, Cell and Environ. 7:371–380.
16. Dieter, P. and D. Marme. 1980. Ca$^{++}$ transport in mitochondrial and microsomal fractions from higher plants. Planta 150:1–8.
17. Drake, M., W.J. Bramlage, and J.H. Baker. 1974. Correlation of calcium content of 'Baldwin' apples with leaf calcium, tree yield, and occurrence of physiological disorders and decay. J. Amer. Soc. Hort. Sci. 99(4):379–380.
18. Elliot, D.C., S.M. Batchelor, R.A. Cassar, and N.G. Marinos. 1983. Calmodulin binding drugs affect responses to cytokinin, auxin and gibberellic acid. Plant Physiol. 72:219–224.
19. Faust, M. and C.B. Shear. 1968. Corking disorders of apples: A physiological and biochemical review. Bot. Rev. 34:441–469.
20. Faust, M.I. and C.B. Shear. 1972. The effect of calcium on respiration of apples. J. Amer. Soc. Hort. Sci. 97(4):437–439.
21. Fukumoto M. and K. Nagai. 1982. Effects of calmodulin antagonists on the mitochondrial and microsomal Ca$^{++}$ uptake in apple fruit. Plant Cell Physiol. 23:1435–1441.
22. Hanson, J.B. 1983. The roles of calcium in plant growth, p. 1–24. In: D.D. Randall, D.G. Blevins, and R. Larson (eds.). Current topics in plant biochemistry and physiology, vol. 1. Univ. of Missouri, Columbia.
23. Haug, A. 1984. Molecular aspects of aluminum toxicity, p. 345-373. In: B.V. Conger (ed.). Critical review in plant Science, vol. 1. CRC Press Inc., Boca Raton, Fla.
24. Hetherington, A. and A. Trewavas. 1982. Calcium-dependent protein kinase in pea shoot membranes. FEBS Let. 145:67–71.
25. Himelrick, D.G. and R.F. McDuffie. 1983. The calcium cycle: uptake and distribution in apple trees. HortScience 18:147–150.
26. Hopfinger, J.A. and B.W. Poovaiah. 1979. Calcium and magnesium gradients in apples with bitter pit. Commun. Soil Sci. and Plant Anal. 10:57–65.
27. Hopfinger, J.A., B.W. Poovaiah, and M.E. Patterson. 1984. Calcium and magnesium interactions in browning of 'Golden Delicious' apples with bitter pit. Scientia Hort. 23:345–351.
28. Jones, R.L. and J.V. Jacobsen. 1983. Calcium regulation of the secretion of -amylase isozymes and other proteins from barley aleurone layers. Planta 158:1–9.
29. Kauss, J. 1983. Volume regulation-activation of a membrane associated cryptic enzyme system by detergent-like action of phenothiazine drugs. Plant Sci. Let. 26:103–109.
30. Kuo, J.F. and P. Greengard. 1969. Cyclic nucleotide-dependent protein kinases. IV. Widespread occurrence of adenosine 3',5'-monophosphate-dependent protein kinase in various tissues and phyla of the animal kingdom. Proc. Natl. Acad. Sci. USA 64:1349–1355.
31. Lee, J.S., T.J. Mulkey and M.L. Evans. 1983. Reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators. Science 220:1375–1376.
32. Leopold, A.C., B.W. Poovaiah, R.K. Dela Fuente, and R.J. Williams. 1974. Regulation of growth with inorganic solutes, p. 780–788. In: Plant growth substances. Hirokawa Pub. Co., Tokyo.
33. Lloyd. C.W. (ed). 1982. The cytoskeleton in plant growth and development, p. 457. Academic Press, New York.
34. Lukas, T.J., D.B. Iverson, M. Schleicher, and D.M. Watterson. 1984. Structural characterization of a higher plant calmodulin: *Spinach oleracea*. Plant Physiol. 75:788–795.
35. Marinos, N.G. 1962. Studies on sub-microscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. Amer. J. Bot. 49:834–841.
36. Mason, J.L. 1976. Calcium concentration and firmness of stored 'McIntosh' apples increased by calcium chloride solution plus thickner. HortScience 11(5):504–505.
37. Marme, D. 1982. the role of Ca$^{++}$ in signal transduction of higher plants, p. 419–426. In: P.F. Waring (ed.). Plant growth substances. Academic Press, New York.
38. Marme, D. and P. Dieter. 1983. Role of Ca$^{++}$ and calmodulin in plants, p. 263–311. In: W.Y. Cheung (ed.). Calcium and cell function, vol. IV. Academic press, New York.
39. Mettler, I.J. and R.T. Leonard. 1979. Ion transport in isolated protoplasts from tobacco suspension cells. I. General characteristics. Plant physiol. 63:183–190.
40. Muto, S. and S. Miyachi. 1977. Properties of a protein activator of NAD kinase from plants. Plant Physiol. 59:55–60.
41. Paliyath, G. and B.W. Poovaiah. 1984. Calmodulin inhibitor in senescing apples and its physiological and pharmacological significance. Proc. Natl. Acad. Sci. USA 81:2065–2069.
42. Paliyath, G. and B.W. Poovaiah. 1985. Identification of naturally occurring calmodulin inhibitors in plants and their effects on calcium- and calmodulin-promoted protein phosphorylation. Plant and Cell Physiol. 26:201–209.
43. Paliyath, G., B.W. Poovaiah, G.R. Munske, and J.A. Magnuson. 1984. Membrane fluidity in senescing apples: effects of temperature and calcium. Plant and Cell Physiol. 25:1083–1087.
44. Poovaiah, B.W. 1979. Effects of inorganic cations on ethephon-induced increases in membrane permeability. J. Amer. Soc. Hort. Sci. 104(2):164–166.
45. Poovaiah, B.W. 1979. Role of calcium in ripening and senescence. Commun. Soil Sci. and Plant Anal. 10:83–88.
46. Poovaiah, B.W. and A.C. Leopold. 1973. Deferral of leaf senescence with calcium. Plant Physiol. 52:236–239.
47. Poovaiah, B.W. and A.C. Leopold. 1973. Inhibition of abscission by calcium. Plant Physiol. 51:848–851.
48. Poovaiah, B.W. and A.C. Leopold. 1974. Hormone-solute interactions in the lettuce hypocotyl hook. Plant Physiol. 54:289–293.
49. Poovaiah, B.W. and A.C. Leopold. 1976. Effects of inorganic solutes on the binding of auxin. Plant Physiol. 58:783–785.
50. Poovaiah, B.W. and A.C. Leopold. 1976. Effects of inorganic salts on tissue permeability. Plant Physiol. 58:182–185.
51. Poovaiah, B.W. and G.A. Moulton. 1982. Vacuum pressure infiltration process for fresh produce. U.S. Patent 4,331, 691.
52. Poovaiah, B.W. and H.P. Rasmussen. 1973. Effects of calcium, 2-chloroethylphosphonic acid, and ethylene on bean leaf abscission. Planta 113:207–214.
53. Poovaiah, B.W. and H.P. Rasmussen. 1973. Calcium distribution in the abscission zone of bean leaves: electron microprobe X-ray analysis. Plant Physiol. 52:683–684.
54. Raghothama, K.G., Y. Mizrahi, and B.W. Poovaiah. The effect of calmodulin antagonists on auxin-induced elongation. Plant Physiol. (In press)
55. Rasmussen, H. 1981. Calcium and cAMP as synarchic messengers. Wiley, New York.
56. Rasmussen, H. and P.G. Barrett. 1984. Calcium messenger system: An integrated view. Physiol. Rev. 64:938–984.
57. Roux, S.J. and R.D. Slocum. 1982. Role of calcium in mediating cellular functions important for growth and development in higher plants, p. 409–453. In: W.Y. Cheung, ed. Calcium and cell function, vol. III. Academic Press, New York.
58. Schleicher, M., D.B. Iverson, L.J. Van Eldik, and D.M. Watterson. 1982. Calmodulin, p. 85–106. In: The cytoskeleton in plant growth and development. Academic Press, New York.
59. Schleicher, M., T.J. Lukas, and D.M. Watterson. 1983. Further characterization of calmodulin from barley (*Hordeum vulgare*). Plant Physiol. 73:666–670.
60. Scott, K.J. and R.B.H. Wills. 1975. Postharvest application of calcium as a control for storage breakdown of apples. HortScience 10(1):75–76.
61. Sharples, R.O. and D.S. Johnson. 1976. Postharvest chemical treatments for the control of storage disorders of apples. Ann. Appl. Biol. 83:157–167.
62. Shear, C.B. 1975. Calcium-related disorders of fruits and vegetables. HortScience 10(4):361–365.
63. Siegel, N. and A. Haug. 1983. Aluminum interaction with calmodulin: evidence for altered structure and function from optical and enzymatic studies. Biochem. Biophys. Acta. 744:36.
64. Suwwan, M.A. and B.W. Poovaiah. 1978. Association between elemental content and fruit ripening in rin and normal tomatoes. Plant Physiol. 61:883–885.
65. Trewavas, A. 1976. Post-translational modification of proteins by phosphorylation. Ann. Rev. Plant Physiol. 27:349–374.
66. Veluthambi, K. and B.W. Poovaiah. 1984. Calcium-promoted protein phosphorylation in plants. Science 223:167–169.

67. Veluthambi, K. and B.W. Poovaiah. 1984. Calcium and calmodulin-regulated phosphorylation of soluble and membrane proteins from corn coleoptiles. Plant Physiol. 76:359–365.

68. Veluthambi, K. and B.W. Poovaiah. 1984. Polyamine-stimulated phosphorylation of proteins from corn (*Zea mays* L.) coleoptiles. Biochem. Biophys. Res. Commun. 122:1374–1380.

69. Williamson, R.E. and C.C. Ashley. 1982. Free Ca$^{++}$ and cytoplasmic streaming in algae *Chara*. Nature 296:647–651.

70. Wilson, R.H. and R.J. Graesser. 1976. Ion transport in plant mitochondria. Encyl. Plant Physiol., New Ser. 3:377–397.

71. Watterson, D.M., D.B. Iverson, and L.J. Van Eldik. 1980. Spinach calmodulin: Isolation, characterization and comparison with vertebrate calmodulins. Biochemistry 19:5762–5768.

# Interspecific Hybridization in the Common Bean: A Review

**Pierre Hucl and Graham J. Scoles**
*Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0*

*Additional index words.* cytogenetics, introgression, species relationships, reproductive barriers, embryo culture

***Abstract.*** **The uses of related wild and domesticated species in the improvement of the common bean, *Phaseolus vulgaris* L., are examined. Obstacles to gene transfer between species and the approaches used in circumventing these barriers are discussed.**

**P. Hucl**

**G.J. Scoles**

The common bean is affected by at least 18 diseases and insect pests in North America (5). The detrimental effects of anthracnose (*Collectotrichum lindemuthianum* Sacc. & Magn. Scrib.), common blight (*Xanthomonas phaseoli* E.F. Sm. Dows.), root rot complexes (*Fusarium solani* Schlecht. f. *phaseoli, Phythium ultimatum* and *Rhizoctonia solani*), white mold (*Sclerotinia sclerotiorum*), and air pollution injury are considered to be of economic importance (69).

The use of intraspecific sources of pest resistance has resulted in the development of anthracnose and virus resistant common bean cultivars. Although attempts have been made to develop blight, root rot, and white mold tolerant bean cultivars, these efforts have met with varying degrees of success to date. The failure of intraspecific hybridization to increase the tolerance of common beans to diseases such as white mold and, to a lesser extent, common blight and root rots has led to the evaluation of related *Phaseolus* species as sources of tolerance or resistance.

A number of desirable physiological traits also have been identified in a number of wild species. Some of the traits of interest and the species in which they have been observed are listed in Table 1. In addition, *P. vulgaris* var. *aborigineus* is immune or tolerant to 4 races of anthracnose (68) as are accessions of *P. coccineus*, *P. ritensis*, and *P. filiformis* (41). Ibrahim and Coyne (44) speculated that the cross-pollination mechanism of *P. coccineus* might be transferred to *P. vulgaris* in order to develop intermating populations for the introduction of new gene complexes and the breakage of existing linkage groups. The complex inheritance of the cross-pollination mechanism in addition to the possibility of selective gene elimination (57) indicate that this will be a difficult task.

## Taxonomy and evolution of *Phaseolus*

For efficient interspecific gene transfer, it is necessary to have information concerning the cytogenetics, origin, and phytogeographical relationships of a cultivated species and

---

Received for publication 5 Apr. 1984. We would like to thank the reviewers for their constructive criticism.The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.