Role of Calcium in Plant Responses to Stresses: Linking Basic Research to the Solution of Practical Problems

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There is ample evidence for the role of Ca in plant growth and development and in maintenance and modulation of various cell functions (Hanson, 1984; Kirby and Pilbeam, 1984; Leonard and Hepler, 1990; Pooviah and Reddy, 1993). This evidence is based on the well-recognized importance of Ca⁺ in membrane structure and function as well as in cell wall structure. For example, it is essential to have Ca⁺ in the extracellular solution to ensure the maintenance of selective permeability, i.e., membrane integrity. It is also well known that Ca⁺ is an integral part of the cell wall where it provides stable, but reversible, intramolecular linkages between pectic molecules, resulting in cell wall rigidity. In addition, Ca⁺ stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids at the membrane surface (Legge et al., 1982). Presence of extracellular Ca⁺ increases bonds between the cell wall and plasma membrane (see Gomez-Lepe et al., 1979). Interestingly, Ca⁺ is a nontoxic mineral nutrient and plant cells can tolerate high concentrations of extracellular Ca⁺ (Palta and Lee-Stadelman, 1983).

In addition to its role in the cell wall and membrane, Ca⁺ is now regarded as an important intracellular secondary messenger (for details, see Pooviah and Reddy, 1993). Various studies have provided strong evidence implicating the regulation of various cell functions by cytosolic (free) Ca⁺ concentration. These studies suggest that Ca⁺ is a messenger in transducing external stimuli in plants. These signals often use plasma membrane-associated protein kinases, phosphatidylinositol pathways, or both (Pooviah and Reddy, 1987).

Many environmental and hormonal signals [touch, wind, gravity, light, cold, auxin, gibberellic acid (GA), abscisic acid (ABA), salt, fungal elicitors] induce changes in cytosolic Ca⁺ levels that precede the physiological responses (for details, see Pooviah and Reddy, 1993). Thus, the impact of environmental and biotic stresses on plants can be mediated by cytosolic Ca⁺. For example, recent evidence suggests such a role of Ca in cold acclimation (Dhindsa and Monroy, 1994; Monroy et al., 1993). In addition, environmental stresses, such as heat, freezing temperatures, and salinity, and biotic stresses are known to cause perturbation in cell membranes, cell walls, or both. Therefore, Ca could be expected to play an important role in plant responses to environmental and biotic stresses. In this article, I illustrate this role of Ca in three stresses: freezing, biotic, and heat.

Examples from our own studies show that, in addition to providing insight into mechanisms of plant responses to stresses, we were able to develop practical solutions to stress-related problems. Our results suggest that manipulation of membrane, cellular, and tissue Ca may be an approach to bridging the gap between science and technology.

ROLE OF CALCIUM IN FREEZING STRESS

Enhanced ion efflux following freeze–thaw stress is mitigated by extracellular Ca⁺

Frozing injury results in increased efflux of ions from plant tissues (Palta et al., 1977a, 1977b). Potassium is the major cation that leaks out of cells (Palta et al., 1977b). From these results, it was suggested that alteration in the K⁺ permeability of the cell membranes was an early symptom of freeze–thaw injury (Palta and Li, 1978, 1980). In a follow-up study, Arora and Palta (1989) demonstrated that K⁺ efflux rate following freeze–thaw injury is markedly reduced in the presence of extracellular Ca⁺. For example, in freeze–thaw–injured onion (Allium cepa L.) bulb cells, K⁺ efflux rate was reduced by half in the presence of 20 mM CaCl₂ in the bathing medium as compared to distilled-deionized water (Table 1). In a related study, K⁺ efflux from onion bulb tissue was reduced by half with 25 mM extracellular CaCl₂, whereas equivalent concentrations of NaCl had no effect on K⁺ efflux (Arora and Palta, 1986). These results suggest that Ca⁺ is able to mitigate freeze-induced injury to the membranes that is responsible for leakage of K⁺.

Protoplasmic swelling as a microscopic symptom of freezing injury: It can be simulated by bathing tissue in K⁺ and prevented by Ca⁺

As discussed above, freeze–thaw stress results in enhanced ion (K⁺) efflux from the cells. In spite of this dramatic change in cell membrane permeability, the cells remain viable, i.e., they can be plasmolysed, they exhibit protoplasmic streaming, and they can be stained with fluorescent dyes (Arora and Palta, 1988; Palta et al., 1977a). Depending on the extent of freeze–thaw stress, these cells either recovered or injury progressed with time during the post-thaw period (Arora and Palta, 1991; Palta et al., 1977b). During the progress of injury, the first visual microscopic system detected was protoplasmic swelling (Palta and Li, 1980).

To examine this symptom further, Arora and Palta (1988) used red onions in which the vacuole and cytoplasm are easily visualized because the vacuole is red and the cytoplasm colorless (Fig. 1). Since cytoplasm occupies <10% of the total cell volume, it can only be recognized as a thin curving over the red vacuole in nonfrozen control cells. This was true in the normal (Fig. 1a) and plasmolysed conditions (Fig. 1b). However, in freeze–thaw–injured cells, the vacuole retracted as a result of protoplasmic swelling (Fig. 1c). Protoplasmic swelling was seen easily when these cells were plasmolysed (Fig. 1d). Palta and Li (1980) proposed that this cellular symptom results from the secondary injury caused by a high concentration of extracellular K⁺, which is present in freeze–thaw–injured cells. We also proposed that injury results when extracellular K⁺ removes Ca⁺ from the outer face of the plasma membrane. This removal causes “weakness” in the structure of the plasma membrane. Support for this notion also came through displacement of membrane Ca⁺ by extracellular Na⁺ (Cramcr et al., 1985). Later, Arora and Palta (1986) demonstrated that protoplasmic swelling can be produced within 30 min by bathing normal cells in 50 mM KCl. Furthermore, they showed that the development of these symptoms by KCl could be prevented by adding 10 to 20 mM CaCl₂ to the bathing medium. These results further support the idea that by maintaining a certain level of Ca⁺ in the membrane, one can mitigate the injury due to freeze–thaw and alkalinity (Na⁺) stresses.

Table 1. Effect of extracellular CaCl₂ on K⁺ efflux rate in control and freeze–thaw–injured onion scale tissue (source: Arora and Palta, 1989).

<table>
<thead>
<tr>
<th>K⁺ efflux rate in the presence of</th>
<th>20 mM CaCl₂</th>
<th>Distilled-deionized water</th>
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<tr>
<td>Frozen</td>
<td>14.8 ± 1.6</td>
<td>8.1 ± 0.3</td>
</tr>
<tr>
<td>No control</td>
<td>47.2 ± 5.0</td>
<td>24.6 ± 2.3</td>
</tr>
<tr>
<td>Ycs (11-11.0C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Calculated as percent total cellular K⁺.</td>
<td></td>
<td></td>
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<tr>
<td>*Mean of three replications ± s.s.</td>
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Loss of membrane-associated Ca$^{2+}$: A key process in the initiation and progression of freezing injury

Using chlorotetacycline (CTC) as a fluorescent probe for membrane-associated Ca$^{2+}$, Arora and Palta (1991) demonstrated that a specific loss of membrane Ca$^{2+}$ occurred following reversible (recoverable) and irreversible freezing injury (Fig. 2). There was a direct relationship between ion leakage and membrane-associated Ca$^{2+}$ (Fig. 3). Injured cells with a possibility of recovery showed significant loss of membrane Ca$^{2+}$ (Fig. 2). These results suggest that loss of membrane Ca$^{2+}$ is associated with early events of freezing injury. Further, these results lend support to the notion that one way to mitigate the injurious impact of freeze–thaw stress is to enhance the ability of the cell to maintain a certain level of membrane Ca$^{2+}$.

Practical applications

Pretreatment with extracellular Ca$^{2+}$ enhances tolerance to freeze–thaw stress. In support of the above discussion, we have found that pretreatment of onion bulb scale tissue with 20 mM CaCl$_2$ before freezing enhanced its tolerance to freeze–thaw stress as measured by the tetrazolium chloride (TTC) reduction assay (Table 2), whereas pretreatment of this tissue with 1 mM ethylene glycol tetracetic acid (EGTA) reduced its tolerance. Since EGTA is known to remove (chelate) Ca$^{2+}$, these results support the view that by maintaining certain levels of Ca$^{2+}$ in the tissue, one may be able to enhance its tolerance to freeze–thaw stress. The protective effect of Ca$^{2+}$ in freezing stress also was demonstrated by Pomeroy and Andrews (1985), who found that wheat (Triticum aestivum L.) cell cultures treated with Ca$^{2+}$ survived much better following ice encasement than the nontreated cultures. Thus, it would appear that, on a practical level, one should be able to improve freezing tolerance by increasing Ca concentration of the tissue. In other words, in a field situation, we can expect to improve freezing tolerance with Ca$^{2+}$ fertilization. We have to be careful, however, in making such a recommendation—the response from supplemental Ca$^{2+}$ application will vary with soil type, available soil Ca$^{2+}$, and type of crop. Obviously, we can expect better response by Ca$^{2+}$ fertilization in tissues and plants that are otherwise deficient in Ca$^{2+}$.

ROLE OF CALCIUM IN BIOTIC STRESSES

Potato tuber quality and Ca$^{2+}$

Bacterial soft rot of potatoes (Solanum tuberosum L.) during storage, caused by Erwinia carotovora pv. atroseptica, decreases as tissue Ca concentration increases (McGuire and Kelman, 1984, 1986). Depending on the cultivar and storage condition, 4% to 38% of stored potatoes can be infected (Varnes et al., 1985). In addition, the incidence of several disorders of tubers, such as internal brown spots, hollow heart, and brown center, also decreases as tissue Ca concentration increases (Collier et al., 1978; Tzeng et al., 1986). Our recent studies have confirmed the beneficial effect of supplemental Ca fertilization on tuber quality (Tawfik and Palta, 1992a, 1992b). Thus, significant improvement in tuber quality and storability can be made by increasing the Ca content of tubers.

Tubers, low-transpiring organs, are naturally deficient in Ca

Since Ca moves along with water in the xylem, transpiration is the main driving force for Ca transport in plants (Clarkson, 1984). Potato tubers, being surrounded by moist soil, will have much less transpiration as compared to the above-ground part of the plant. Consequently, low-transpiring organs, such as fruits and tubers, will accumulate much less Ca per unit fresh weight than leaves. Calcium deficiency in tuber tissue is even greater for potatoes grown in sandy soil, such as in central Wisconsin, the major potato-growing area of the state, because of the low level of exchangeable Ca$^{2+}$ in these soils. Moreover, with constant irrigation, water-soluble Ca$^{2+}$ will be depleted from the top of the hill where most of the tubers form.
Fig. 2. Photomicrographs of adaxial epidermal cells of onion bulb (nonfrozen control and freeze-thaw-stressed) treated with fluorescein diacetate (FDA) and chlormeracycline (CTC) staining solutions. (a) Control cells showing fluorescence from FDA staining; (b) freeze-thaw-stressed (~11.5C) cells showing fluorescence from FDA staining; (c) control cells exhibiting Ca²⁺-CTC fluorescence; (d) bright field picture of (c); (e) freeze-thaw-stressed (~8.5C) cells showing Ca²⁺-CTC fluorescence; (f) bright field picture of (e); (g) freeze-thaw-stressed (~11.5C) cells showing Ca²⁺-CTC fluorescence; (h) bright field picture of (g). CW = Cell wall; P = protoplasmic surface (plasma membrane). Magnification in (a) and (b) was ×180 and in (c) to (h) ×500 (source: Arora and Paul, 1988).
Fig. 3. Relationship between freezing injury (ion leakage) and membrane-associated Ca\(^{2+}\)-chlorendecycline (CTC) fluorescence (mean ± SE) (source: Arora and Palla, 1988).

Table 2. Effect of CaCl\(_2\) and a 2-h ethylene glycol tetraacetic acid (EGTA) pretreatment on cell viability of onion scale tissue after freeze–thaw stress. Cell viability was assessed by the tetrazolium chloride (TTC) reduction method (Palla et al., 1981) (source: Arora and Palla, 1989).

<table>
<thead>
<tr>
<th>Freezing temp (°C)</th>
<th>TTC reduction (%)</th>
<th>CaCl(_2) (mM)</th>
<th>EGTA (mM)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td>None (control)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>-3</td>
<td>37.6 ± 4.2*</td>
<td>79.5 ± 8.3</td>
<td>28.6 ± 2.7</td>
</tr>
<tr>
<td>-5</td>
<td>11.6 ± 1.3</td>
<td>24.2 ± 3.1</td>
<td>13.5 ± 1.6</td>
</tr>
<tr>
<td>-7</td>
<td>8.0 ± 0.9</td>
<td>6.3 ± 0.5</td>
<td>10.0 ± 0.8</td>
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*Mean of six replications ± SE.

Discovery of tuber roots: Application of soluble Ca\(^{2+}\) around the tuber area can enhance tuber Ca\(^{2+}\) uptake

Over 10 years ago, we provided evidence for the existence of functional roots on the tuber (Fig. 4a) and at the tuber–stolon junction (Kratzke and Palla, 1985). In a follow-up study, we showed that these tuber roots displayed normal root anatomy; also, they appear to derive from parenchyma cells adjacent to the vascular tissue (Struckmeyer and Palla, 1986). By feeding a water-soluble dye, we demonstrated that these roots were able to supply water to the tuber (Fig. 4c and d), whereas the main root system supplied water to the top part of the plant (Fig. 4b). Since water and Ca are known to move together, we suggested that these tuber and stolon roots are able to supply Ca to the tuber (Kratzke and Palla, 1985). Using a divided-pot system, where supplemental Ca\(^{2+}\) could be applied either in the tuber area or to the main root system, Kratzke and Palla (1986) found that adding Ca\(^{2+}\) to the main root system increased Ca\(^{2+}\) concentration of tuber tissue (Fig. 5). However, applying Ca\(^{2+}\) to the tuber and stolon area resulted in a 3-fold increase in Ca\(^{2+}\) concentration in the tuber peel and medullary tissue (Fig. 5). These results showed that tuber Ca\(^{2+}\) content can be increased by placing Ca\(^{2+}\) in the tuber and stolon area. Thus, on a practical level, these results indicated that placement of Ca\(^{2+}\) is important for enhancing Ca\(^{2+}\) uptake by the tuber. In support of these studies, Simmons and Kelling (1987) and Simmons et al. (1988) found that maximum increase in tuber Ca\(^{2+}\) occurred when applied Ca was mixed in the hill where tubers develop.

Spoon feeding potatoes during bulking: A new concept in potato nutrition

Our discovery of tiny roots on tubers (Fig. 4a) has changed the concept of potato nutrition. Previously, it was believed that the potato plant’s main roots supplied all the water and nutrients to the leaves, and the leaves in turn feed the tubers. In contrast, our results clearly show that potato tubers are like “underground plants” that draw their water and nutrients, such as Ca, directly from the soil. Since tubers are surrounded by moist soil, they cannot compete with leaves for transpiration.

Rational water uptake. Tubers thus have to rely on roots that are in their close proximity (tuber roots, tuber–stolon junction roots, stolon roots) to extract water from the soil. Since Ca moves in xylem along with water, it follows that potato tubers must extract Ca from the soil in their close proximity. These results have lead to the development of a concept of “spoon feeding” Ca to the tubers during the bulking period.

Practical applications

Three practical questions, for enhancing tuber quality through tuber Ca nutrition, have been addressed by our research: 1) where to apply Ca (i.e., placement); 2) when to apply Ca (i.e., timing); 3) what source of Ca is most effective.

1) Placement. Our results show that tubers take up Ca from the soil surrounding the tuber and stolon area. Thus, to enhance Ca concentration in the tubers, it is important that Ca be added in the upper portion of the hill where tubers develop.

2) Timing. Because tubers develop late in the season, it is important to add supplemental Ca during bulking, which is even more critical in sandy soils. Due to low moisture-holding capacity, sandy soils are irrigated two to three times a week. Thus, the top portion of the hill is continuously washed by irrigation and rain, with water moving soluble nutrients to the lower portion of the hill. These nutrients remain accessible to vegetative growth via the main root system. However, the tubers developing during late season will not have access to these nutrients via the tuber or stolon roots. Thus, to enhance tuber Ca uptake, we need to “spoon feed” potatoes during bulking with Ca fertilizer. Before our research, potato growers used to complete fertilization at hilling. This was a necessity, because nutrients could not be applied by tractor after hilling without damaging the plants. Our results show that Ca needs to be made much later in the season and that this can be easily achieved by injecting Ca fertilizer directly into the irrigation line (Tawfik and Palla, 1992a).

3) Source and quantity. We have found that Ca should be applied in water-soluble form to facilitate uptake by the tuber. Common sources of Ca used in agriculture are lime and gypsum. These chemicals have low water solubility. Because we are injecting the fertilizer into the irrigation line, we have used two water-soluble sources of Ca available in the market—calcium nitrate (Hydro Agri of North America) and NHIH (Stoller Chemical Co.). We have injected these two products separately and found them to be effective in increasing tuber Ca (Tawfik and Palla, 1992a). Applications were made during tuber bulking in three split doses (hilling and 3 and 6 weeks after hilling). Our results demonstrate that it is possible to improve tuber Ca content and, thereby, tuber quality by applying 113 to 226 kg Ca/ha during bulking, even in soil that contained sufficient Ca for potato plant growth.

ROLE OF CALCIUM IN HEAT STRESS

Heat stress results in perturbation of cell membrane transport properties. It can be detected as enhanced ion leakage from the cells (Palla et al., 1981). Extracellular Ca reduces membrane leakage at elevated temperatures (Cooke et al., 1986; Toprover and Glinka, 1976). These results suggest that Ca may be able to protect plants from heat stress. Recently, we have found evidence in support of this suggestion (Tawfik and Palla, 1992b). We studied the impact of Ca on potato plant growth and tuber production during heat stress. These results not only provide some insight into the physiological role of Ca in heat stress, but also provide an avenue for mitigating heat stress on plants in the field.

Potato: A cool-season crop sensitive to heat stress

Heat stress reduces potato plant growth and partitioning of photosynthesate to the tubers (Tawfik, 1981). Although there are differences among cultivars in their response to heat stress, in general, day maxima more than 25 to 30°C tend to increase stem length and branches while reducing leaf size and total leaf area (Benoit et al., 1983; Khedher and Ewing, 1985; Manrique, 1990). These morphological changes dramatically reduce the leaf : stem ratio under heat stress (Bodlaender, 1963; Burton, 1989; Khedher and Ewing, 1985; Steward et al., 1981).
In addition, high temperatures also reduce the net assimilation rate (Midmore and Prange, 1992; Steffen et al., 1995). Overall, the result of all these effects of heat stress on potato plants is a decrease in plant growth and tuber yield.

**Heat stress effects on potatoes can be mitigated by Ca**

Summer 1988 was unusually warm and dry in Wisconsin. The daily air maxima temperature between 1 June and 18 Aug. at the Hancock Experimental Station was at or above 32°C for 46 days. The rainfall during June, July, and August was 47, 140, and 100 mm, respectively, at this location. On average, there was a 25% decrease in tuber yield in the central sands (where Hancock Experimental Station is located) of Wisconsin. In our field trials at Hancock, we found a 20% to 30% increase in tuber yields when soluble Ca (calcium nitrate or N-HIB) was applied during the tuber bulking period (Fig. 6). There was no significant difference in tuber yield among the two sources of Ca. These results suggest that Ca fertilization during

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**Fig. 4.** Evidence for the existence of functional roots on 'Russell Burbank' potato tubers and stolons and study of the transport of water-soluble dye from various roots to the tuber. (a) Growing tubers with tuber roots; (b) dye given to main roots is transported to the leaves and not to the tubers; (c) feeding the dye to stolon roots; (d) movement of dye from stolon roots to the tuber (source: Kratzke and Pala, 1985).

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**Fig. 5.** Tuber Ca concentration as influenced by placement of water-soluble Ca. Fertilizer (CaCl₂) was selectively given to the main roots or to the tuber and stolon in divided pot system (source: Kratzke and Pala, 1986).

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**Fig. 6.** Impact of Ca and N application, during tuber bulking period, on potato tuber yield under heat stress conditions. Field plots were established in 1988 at the Univ. of Wisconsin Hancock Experimental Station. All plots were irrigated with a central pivot system and were grown under standard fertility and cultural practices. Calcium application was made by injecting water-soluble forms of fertilizers (calcium nitrate or N-HIB) into the irrigation line. Values are means of five separate locations for yield measurement.
bulking could mitigate the adverse impact of heat stress on tuber yield.

Following these observations in 1988, we have conducted several studies on the impact of heat stress on potato plants under controlled environmental conditions (Tawfik and Palta, 1992b). In these studies, side-by-side comparisons were made on the impact of Ca nutrition on potato plants grown at either 20°C/day/15°C night cycles (normal) or 30/15°C (heat stress). Plants were grown in a sandy loam soil that tested at 1300 kg/m³ available Ca (adequate amount of Ca for potato growth). Plants under heat stress had reduced total leaf fresh and dry weights compared to the control. However, plants receiving Ca under heat stress had significantly higher total leaf fresh and dry weights compared to plants with no supplemental Ca under identical conditions. This beneficial effect of Ca was absent under nonstress conditions. In addition, under heat stress, plants receiving supplemental Ca had higher leaf Ca contents and higher stomatal conductance. These results show that applying Ca during heat stress can mitigate heat stress effects and that maintaining a certain level of Ca in leaf tissue is important under heat stress.

**Physiological basis for mitigation of heat stress effects on potatoes**

Although we do not know the mechanism by which Ca is able to mitigate heat stress effects on potatoes, our results provide some insight. For example, we found that stomatic conductance was higher in Ca-treated than control plants under heat stress (Tawfik and Palta, 1992b). This result would be relevant in avoiding heat stress effects via enhanced transpiration and water loss. There is some evidence for the regulation of stomatal function by cytosolic calcium Ca²⁺ (Irving et al., 1992; Schwartz et al., 1988), although the exact mechanism remains to be elucidated (Kears and Assmann, 1993). We found a decrease in the Ca concentration in leaves of potato plants exposed to heat stress, but the Ca²⁺ concentration was maintained at the same level as before heat stress in the leaves of plants given Ca fertilization during heat stress (Tawfik and Palta, 1992b). Our results suggest that maintaining a certain Ca²⁺ level during heat stress is essential for normal stomatal function. Additionally, plasma membrane H⁺-ATPases are one of the effectors of stomatal opening (see Kears and Assmann, 1993) and their activity is regulated by Ca-stimulated protein kinase (Schaller and Sussmann, 1987). Thus, Ca²⁺ possibly can regulate stomatal function by regulating H⁺-ATPase activity in the guard cells. If this is true, then, by maintaining a certain Ca level in the guard cell, it may be possible to maintain normal stomatal functioning, which, in turn, would allow plants to avoid heat stress effects by dissipating heat through transpiration.

**Literature Cited**


