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Root zone calcium modulates the response of potato plants to heat stress

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Potato plant growth and development are known to be severely impacted by heat stress. Here plants grown in a chemically inert medium of 1 : 1 quartzite : perlite (v : v) were subjected to either 35/25°C (stress) or 20/15°C (control) day/night air temperatures and four concentrations of root zone calcium (5, 25, 125 and 600 μ*M* Ca) for 3 weeks. We report for the first time that potato plant growth under heat stress can persist at specific levels of Ca^{2+} in the root zone and that the Ca^{2+} level required for growth under heat stress exceeds that required for growth under normal temperatures. We also provide strong, initial evidence that the ability of high Ca^{2+} levels to mitigate heat stress effects results from shifts in meristematic activity. Total foliar mass and leaf area were essentially unaffected by Ca^{2+} level under control temperatures. Under heat stress, leaf area was reduced to about 5% of the control at 5 and 25 μ*M* Ca but to only 70% of the control at 125 and 600 μ*M* Ca. Likewise, total foliar mass was reduced under heat stress to about 30% of the control at 5 and 25 μ*M* Ca but total foliar mass was greater under heat stress than control conditions at 125 and 600 μ*M* Ca. This increase at higher Ca^{2+} concentrations was due primarily to axillary shoot growth. Anatomical studies of leaves grown under heat stress show that cell expansion was impaired by heat stress and this impairment was overcome by increasing root zone calcium levels. These results provide insight into the mechanism by which root zone Ca^{2+} may modulate plant response to heat stress.

Introduction

Heat stress in cultivated potato (*Solanum tuberosum* L.) often manifests as reduced carbon fixation and partitioning to tubers (Ewing 1981, Reynolds and Ewing 1989, Reynolds et al. 1990, Gawronska et al. 1992, Havaux 1994), declines in plant and tuber growth (Ewing 1981, Mendoza and Estrada 1977, Menzel 1985, Palta 1996), and impaired tuber initiation and development (Mendoza and Estrada 1977, Ewing 1981).

The well-documented structural and metabolic roles of calcium (Ca^{2+}) suggest that its availability may specifically impact plant response to temperature stress (Palta 1996). For example, Ca^{2+} contributes to overall cell wall and membrane structural integrity (Sartorius and Weis 1988, Palta 1990, Marschner 1995, Palta 1996, Seling et al. 2000) and cell, tissue, and organ growth (Emanuelsson 1984, Schiefelbein et al. 1992, Miller et al. 1997, White 1998). Loss of membrane or cellular Ca^{2+} in the early stages of injury by environmental stresses has been reported (Arora and Palta 1988, 1989, Palta 1990). Flux in cellular Ca^{2+} has been proposed as a signal that alters gene expression in response to environmental stresses (Bush 1993, Poovaiah and Reddy 1993, Subbaiah et al. 1994a, 1994b, Monroy and Dhindsa 1995, Polisensky and Braam 1996, Liu and Zhu 1997, Gong et al. 1998).

We recently provided the first evidence that root zone Ca^{2+} levels could mitigate the response of potato plants to prolonged heat stress (Tawfik et al. 1996). In that work, the effect of soil Ca^{2+} level on fundamental responses to conditions reflecting naturally occurring heat stress episodes was investigated under controlled environment conditions (Tawfik et al. 1996). Significantly

Abbreviations – DAP, days after transplanting into experimental medium; D/N, day/night; EPG, leaflet adaxial epidermal ground cells; FW, fresh weight; TRT, smallest leaf on the primary shoot axis with a visible terminal leaflet at initiation of calcium and temperature treatment; TRT A3, third leaf apical to TRT leaf; RWC, relative water content.

greater values of leaf tissue Ca^{2+} concentration and membrane thermostability, stomatal conductance, and tuber production were associated with soil Ca^{2+} amendment during heat stress (Tawfik et al. 1996). However, Ca^{2+} concentrations in the root zone were not precisely controlled in previous work (Tawfik et al. 1996). Appropriate and precise methodology in the present study permitted exact maintenance of target root zone Ca^{2+} levels. The primary objective of this study was to investigate changes in potato plant growth following simultaneous exposure to temperature and root zone Ca^{2+} treatment in controlled environments. The influence of root zone Ca^{2+} level on biomass accumulation and partitioning and leaf and stem anatomy was investigated under heat stress and normal temperatures to gain insight on mechanisms by which calcium may modulate their responses.

Materials and methods

Germplasm, growth medium preparation, and transplanting

Micropropagated plantlets of cv. Red Norland with 5–7 nodes were transplanted into one 0.01 m^3 plastic tray containing Jiffy-Mix (JPA, East Chicago, IL, USA) moistened with distilled H_2O. Plants were maintained in a growth chamber for 7 days under 20/15 °C D/N air temperatures, ambient relative humidity, and 400 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) supplied by cool white fluorescent lamps (14 h photoperiod). Plants were irrigated with distilled H_2O during establishment. After 7 days, separate plants were transferred to individual 3.5 l pots containing an inert 1 : 1 (v/v) mixture of medium-grain quartzite (Cherry Stone ≤ 1 Fine, New Ulm Quartzite Quarries, New Ulm, MN 56073, USA) and perlite (Terra-Lite Coarse, Horticultural Products, W.R. Grace and Company, Cambridge, MA 02140, USA) for the remainder of the study. The bulk density, particle density, and porosity of the medium were 0.714 g ml^{-1}, 2.05 g ml^{-1}, and 64%, respectively. The ion retention capacity of the medium was estimated to be 0 meq $(100\ g)^{-1}$ after measuring the electrical conductivity of medium rinsate in preliminary bench-top studies. Before medium mixing and transplanting, the quartzite and perlite were rinsed with distilled H_2O until the electrical conductivity of the rinsate was similar to distilled H_2O (medium was soaked overnight in distilled-deionized H_2O prior to testing electrical conductivity).

At transplanting, 1.8 kg of medium moistened with distilled-deionized (dd) H_2O was placed in each 3.5 l pot. Fine mesh screen in the bottom of each pot prevented medium leakage. Evaporative H_2O loss from the medium was significantly reduced by covering 70% of the medium surface with a circular 17-cm diameter sheet of polyethylene with a circular 5.5-cm diameter hole in the centre. An additional 200 g of moistened medium was spread on the polyethylene sheet. Thirty single plants were removed from the 0.01 m^3 plastic tray, rinsed free of Jiffy-Mix, placed in the centre of a pot (i.e. hole in polyethylene sheet) with 2 nodes exposed, and covered with clear plastic cups for 2 days to minimize transplant shock. Twelve plants were maintained in two identical and adjacent chambers.

Environmental conditions and nutrient solution delivery

Beginning 2 days after transplanting into experimental medium, plants were auto-irrigated to excess several times per day with a complete nutrient solution containing 250 μ*M* Ca from $CaCl_2 \cdot 2H_2O$ and adjusted to pH 6.1 with 1 *M* KOH. Calcium treatment (5, 25, 125, and 600 μ*M* Ca; 6 plants per level) was initiated 22–26 days after transplanting (DAP) into experimental medium. All other nutrients (except Cl) and pH were unchanged from pre-treatment levels. Residual Ca^{2+} in the medium was reduced before initiation of Ca^{2+} treatment by dispensing 750 ml dd H_2O to each pot — the electrical conductivity of the final effluent was similar to dd H_2O. Calcium treatment was initiated by (1) dispensing 750 ml of the appropriate treatment solution to the top of the pot through the hole in the polyethylene sheet soon after effluent from the dd H_2O rinse had stopped exiting the pot due to gravity; and (2) establishing a siphon nutrient solution delivery system described below.

Fresh nutrient solution was delivered continuously to each pot via a siphon established through 0.51 mm or 1.02 mm ID microbore tubing (Tygon Formula S-54-HL, Norton Performance Plastics Corp., Akron, OH, USA). The single line serving each pot was situated so that solution dripped freely into a plastic tube (7 cm long, 1.5 cm inner diameter) embedded 4 cm into the medium (2 cm beneath the polyethylene sheet) at the edge of the hole in the polyethylene sheet 2 cm from the stem at planting. Solutions were held in opaque reservoirs outside each growth chamber (ambient temperature approximately 20°C). All siphon lines were 4.07 m long and pots and reservoirs were at identical vertical positions with reservoir bottoms 30 cm above the ends of the lines serving the pots. Changes in nutrient solution delivery rate were achieved by varying the hydraulic head and/or tubing internal diameter. At all times, however, nutrient solution was delivered in excess of plant need since effluent from each pot was always present. Effluent was collected in basins beneath pots and discarded. Solution delivery rate to plants declined slightly with reductions in the hydraulic head over short time periods but the 4 l reservoirs containing nutrient solution were refilled daily at 12 : 00–14 : 00.

Two days after the initiation of Ca^{2+} treatment, the temperature regimen in one chamber was changed to 35/25°C day/night (stress) while temperatures remained at 20/15°C day/night (control) in the other chamber. Control and stress air temperatures were maintained until harvest 44–49 DAP. Three plants per Ca^{2+} level were arranged in a completely randomized design within each chamber. At the initiation of temperature treatment, the smallest leaf on the primary shoot axis with a visible

terminal leaflet (approximately 2–3 mm long) was tagged and referred to as 'TRT' leaf. At this time, axillary shoots were absent on all plants.

Biomass measures

At harvest, aboveground biomass was grouped into two categories: (1) stems and leaves of the primary axis and (2) stems and leaves of axillary shoots. Leaves approximately 5 mm long were counted. Tissue fresh weight (FW) was recorded immediately after excision and area measures were taken of all leaves soon after FW readings using an area meter (model LI-1600; Li-Cor, Inc., Lincoln, NE, USA). Within 15 min of harvest, tissue was placed in a forced-air oven (65°C, 72 h) prior to being re-weighed.

Anatomical and morphological measures

Tissue of the first replicate (plant) of each Ca^{2+} level (heat-stressed plants only) was retained for evaluation after FW measures at harvest. The section of primary stem apical to the point of attachment of TRT leaf was fixed and cleared in several changes of 100% ethanol at room temperature then cleared further with 75% lactic acid overnight at room temperature. Stem sections were sequentially re-hydrated, exposed overnight to a lignin specific stain (1% pararosanaline hydrochloride), and dehydrated in a graded series of ethanol at room temperature. Dehydration was discontinued when sufficient stain had been removed so that vascular elements were evident to the unaided eye. The distribution and structural continuity of stained vascular elements in stem sections were compared to published reports of the same for potato (McCauley and Evert 1988a).

At harvest, the terminal leaflet of the third leaf apical to TRT leaf (here designated as TRT A3 leaf) was excised, weighed, measured for area, and placed directly into 100% ethanol in preparation for microscopic examination. Tissue sections excised from the middle third of the terminal leaflet, external to the midrib, were examined using scanning electron microscopy (SEM). Three SEM prints (image × 500) taken of the upper surface of each leaflet were used to measure the density of stomatal complexes and epidermal ground cells. With clear plastic sheets overlying SEM prints, all cells in the SEM image were easily identified, marked, and counted. Cells were included in counts if ≥ 25% of the estimated cell area was evident at the margin of the SEM image. Stomatal index (SI) was calculated using the formula of McCauley and Evert (1988b):

SI (%) = [no. stomatal complexes (no. stomatal complexes + no. epidermal ground cells)$^{-1}$] × 100. Average epidermal ground cell area was estimated with the formula:

Average Cell Size (μm^2) = SEM image area (no. epidermal ground cells)$^{-1}$. Also, estimates of the total number of cells in the adaxial epidermis of each leaflet were calculated as:

Total Epidermal Cells = sum epidermal ground cells and stomatal complexes (μm^2) $^{-1}$ × leaflet area.

Statistical analysis

Leaf epidermal cell type distribution

Data were analysed using a model for a completely randomized design in PROC GLM procedure of Statistical Analysis System (SAS, version 6.11, Cary, NC, USA). Due to significant temperature × Ca^{2+} level interactions, means tests (Duncan's Multiple Range, $\alpha = 0.05$) were completed to examine the impact of Ca^{2+} level within temperature regimen (Steel and Torrie 1980).

Results

Heat stress effects on plant growth were mitigated by calcium level

Overall, an increase in Ca^{2+} supply to the root zone significantly increased foliar FW (Fig. 1), total plant leaf area (Fig. 2A), and foliar RWC (Fig. 2B), regardless of temperature regimen. However, we found a clear tendency of higher Ca^{2+} levels to mitigate heat stress effects. Total foliar FW was greater under heat stress than control conditions in plants supplied with 125 and 600 μM Ca (Fig. 1C). Also, leaf area under heat stress was reduced to 5% of control plants at 5 and 25 μM Ca but remained at 70% of control plants at 125 and 600 μM Ca (Fig. 2A). Relative water content was also significantly impacted by Ca^{2+} level, especially under heat stress (Fig. 2B).

Changes in biomass accumulation due to temperature and Ca^{2+} level were accompanied by shifts in biomass partitioning. Most growth in plants subjected to heat stress occurred in axillary shoots and to an extent positively related to Ca^{2+} level (Fig. 1). Overall, axillary shoot FW was approximately 25% and 40% of total foliar FW under control and heat stress conditions, respectively (Fig. 1). More importantly, axillary shoot FW averaged 43% of total foliar FW at Ca^{2+} levels ≥ 125 μM under heat stress but only 5% at the same Ca^{2+} levels under control conditions (Fig. 1). Interestingly, axillary shoot FW constituted nearly the same percentage of total foliar FW at Ca^{2+} levels ≤ 25 μM under control (33%) and heat stress (31%) conditions (Fig. 1). As expected, tuberization was inhibited under heat stress in all plants (data not shown).

Calcium level affected leaf but not stem anatomy under heat stress

Plants provided continuously with a solution containing ≤ 25 μM Ca during heat stress were clearly smaller than plants receiving greater Ca^{2+} levels. The construct of this experiment did not allow for comprehensive study of the possible explanations of this response. Nevertheless, steps were taken to verify whether growth reduc-

tions resulted from physical impedance of nutrient and water flow to apical portions of the primary shoot axis. The impact of Ca^{2+} level on leaf anatomy was also examined.

The intensity, distribution, and continuity of stained vascular elements were similar in stem sections produced during heat stress, regardless of Ca^{2+} level (data not shown). For example, staining patterns in stems of plants supplied with 25 or 600 μ*M* Ca were similar to diagrams of normal *Solanum tuberosum* vascular tissue shown in McCauley and Evert (1988a). Calcium level did not affect stomatal index values and had little impact on the number of stomata per unit area in the adaxial epidermis of terminal leaflets which developed under high temperatures (Table 1). Epidermal ground cell density, however, was significantly and negatively related to Ca^{2+} level (Table 1). Epidermal ground cell density was significantly lower at 125 and 600 compared to 25 μ*M* Ca (Table 1). Average EPG area and total number of cells in the entire leaflet surface was positively related to Ca^{2+} level under heat stress (Table 1). Cells were fewer and smaller in plants supplied with 5 or 25 μ*M* Ca compared to greater Ca^{2+} levels. Interestingly, plants given 125 μ*M* Ca had a high average cell size but moderate estimated cell number (Table 1). Growth at 600 μ*M* Ca resulted in the greatest cell number and size (Table 1). It was also apparent that Ca^{2+} level impacted leaf cell morphology under heat stress. Margins of epidermal ground cells of plants grown with 25 μ*M* Ca under high temperatures were smooth while cell margins in plants supplied with 600 μ*M* Ca were scalloped (Fig. 3).

Discussion

Potato plant and tuber growth normally decline significantly under heat stress (Mendoza and Estrada 1977, Ewing 1981, Menzel 1985, Palta 1996, Tawfik et al.

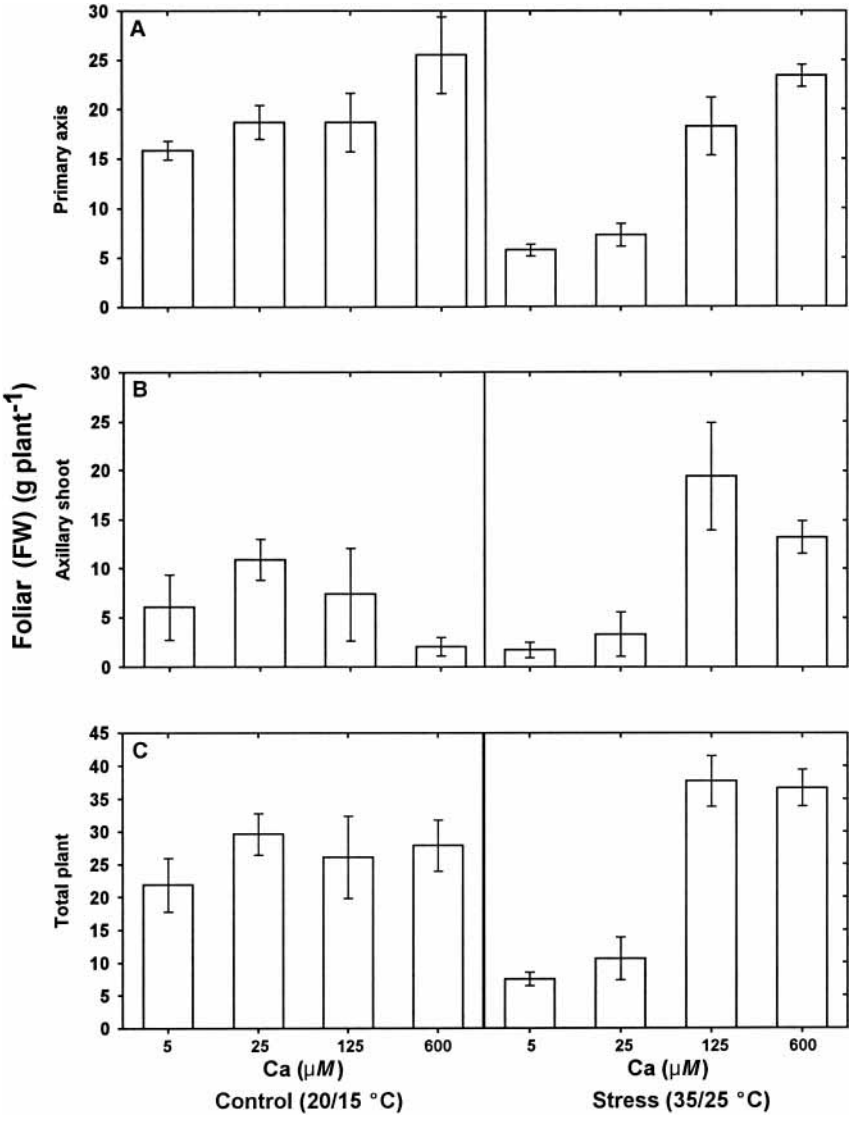

Fig. 1. Temperature and root zone [Ca^{2+}] effects on potato (*Solanum tuberosum* L., cv. Red Norland) plant FW. Plants were continuously supplied with nutrient solution containing the indicated [Ca^{2+}] during exposure to control (20/15°C, day/night) or heat stress (35/25°C, day/night) temperatures (n = 3, ± SD).

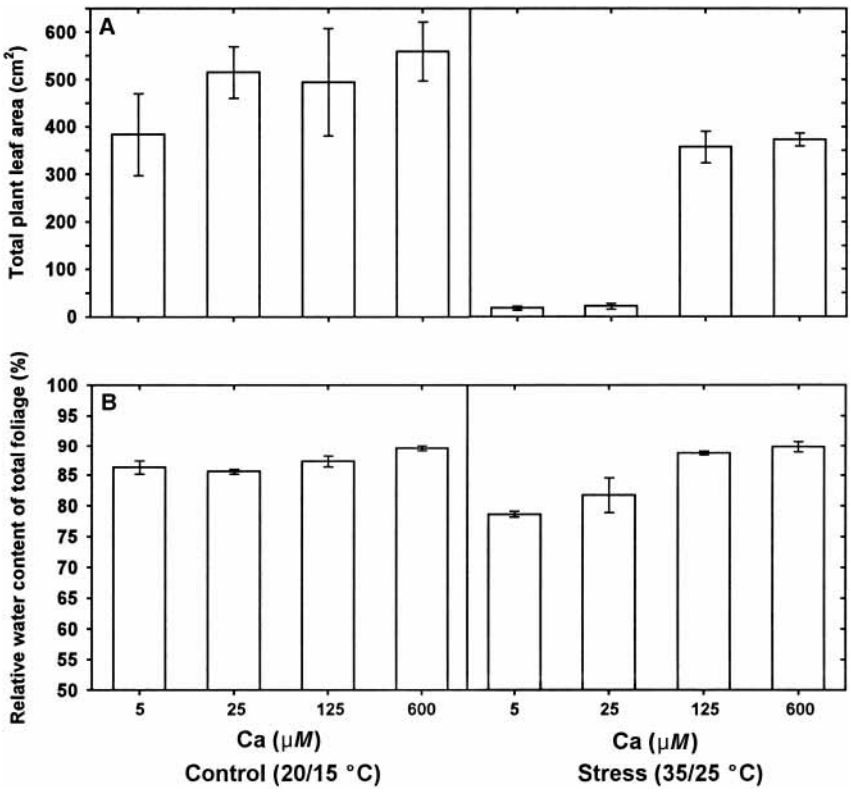

Fig. 2. Temperature and root zone $[Ca^{2+}]$ effects on potato (*Solanum tuberosum* L., cv. Red Norland) plant leaf area (A) and foliar relative water content (B). Plants were continuously supplied with nutrient solution containing the indicated $[Ca^{2+}]$ during exposure to control (20/15°C, day/night) or heat stress (35/25°C, day/night) temperatures (n = 3, ± SD).

1996). However, we report for the first time that potato plant growth under heat stress can persist at specific levels of Ca^{2+} in the root zone and that the Ca^{2+} level required for growth under heat stress exceeds that required for growth under normal temperatures. Using precise methodology, we are the first to document the exact range of root zone Ca^{2+} level over which potato plant growth is impaired or maintained under heat stress. Here a complete nutrient solution containing a fixed level of Ca^{2+} was continuously supplied to a uniform, chemically inert rooting medium. This approach allowed for a reliable description of temperature and Ca^{2+} level effects on plant growth and morphology.

Total plant FW and leaf area were similar at root zone Ca^{2+} levels from 5 to 600 μ*M* under normal temperatures but significantly reduced at less than 125 μ*M* Ca under high temperatures (Fig. 1C, Fig. 2A). Calcium concentrations of 10–100 μ*M* in flowing nutrient solutions have been considered sufficient to maintain plant demand, depending on species, relative growth rate, and developmental stage (Hanson 1984). The lack of a dramatic response to Ca^{2+} in the range of concentration used here (5–600 μ*M*) under normal temperatures is consistent with the early work of Hanson (1984). But, our results show that heat stress effects on potato plant growth were mitigated only at Ca^{2+} concentrations ≥

Table 1. Influence of root zone $[Ca^{2+}]$ during heat stress on morphological variables of adaxial epidermis in potato (*Solanum tuberosum* L., cv. Red Norland) leaf tissue. The smallest leaf of the primary shoot axis present at the initiation of heat stress (35/25°C day/night) was tagged. After 21 days of simultaneous temperature and Ca^{2+} treatment (5, 25, 125, or 600 μ*M*), the terminal leaflet of the third leaf apical to the tagged leaf was examined under scanning electron microscopy (SEM). Area of this terminal leaflet is shown in the final column. Means within the same column followed by a common letter are not significantly different (LSD test, α = 0.05).

	No. cells per unit surface (10^5 μm²)						
	Stomata	Epidermal ground EPG	Total	Stormatal index (%)	Average EPG area (μm²	Total no. cells in leaflet surface	Terminal leaflet area (cm²
Ca treatment (μ*M*)							
5	28.7 b	173.6 b	202.3 b	14.4 a	589 bc	341877 c	1.69
25	38.2 a	234.4 a	272.6 a	14.2 a	432 c	463453 c	1.70
125	27.8 b	134.6 bc	162.4 bc	17.4 a	756 ab	933547 b	5.75
600	26.1 b	127.6 c	153.7 c	16.9 a	784 a	1217097 a	7.92
$LSD_{(0.05)}$	7.6	45.4	44.3	5.0	169	139476	
Pr > F	0.0245	0.0023	0.0010	0.3804	0.0044	< 0.0001	

125 μ*M*. These results are consistent with our conclusion that higher than normal Ca^{2+} levels in the root zone are required to maintain growth under heat stress.

The ability of high Ca^{2+} levels to mitigate heat stress effects is not surprising, given the fundamental roles of Ca^{2+} in structure and physiology (Hanson 1984, Marschner 1995) and in plant responses to environmental stresses (Palta 1996). Furthermore, we reported earlier that supplementing a field soil with nitrogen and calcium mitigated some effects of heat stress on potato plants (Tawfik et al. 1996). In the present study, we provide evidence for the exact range of root zone Ca^{2+} level over which plant growth is inhibited or maintained under heat stress. We also provide strong, initial evidence that the ability of high Ca^{2+} levels to mitigate heat stress effects results from shifts in meristematic activity.

Heat stress normally compromises the function and dominance of the apical meristem, thereby minimizing and altering growth. However, we are the first to document that specific, elevated levels of Ca^{2+} in the root zone under heat stress protect against significant heat stress-induced growth impairment by allowing continued meristematic activity, especially in secondary shoots. Data in Fig. 1 verify that primary apical meristem function is impaired under heat stress and that growth continues to a greater extent in secondary shoots. In fact, extensive axillary shoot growth at Ca^{2+} levels ≥ 125 μ*M* clearly contributed to the fact that total plant FW was greater at these Ca^{2+} levels under heat stress than control temperatures (Fig. 1C).

Reduction in leaf area is also a common manifestation of heat stress in potato (Gawronska et al. 1992). Nevertheless, we provide the first evidence that specific elevated levels of Ca^{2+} in the root zone can mitigate heat stress-induced reductions in leaf area. Increasing the supply of Ca^{2+} to the root zone of plants subjected to heat stress significantly increased total plant leaf area (Fig. 2A). Total plant leaf area under heat stress was reduced to 5% of control plants at 5 and 25 μ*M* Ca but remained at 70% of control plants at 125 and 600 μ*M* Ca (Fig. 2A). It is important to note that mitigation of the effect of heat stress on leaf area occurred only at Ca^{2+} levels exceeding those previously reported to be adequate for normal plant growth (Hanson 1984) or the prevention of Ca^{2+} deficiency-related symptoms in potato leaves of plants grown under normal temperatures (Seling et al. 2000).

The mechanism behind reduction in potato plant growth under heat stress is unclear. Substantial effort has yet to adequately explain why and how potato plants respond to heat stress in characteristic ways. Changes in respiration rate or photosynthetic capability (Dinar and

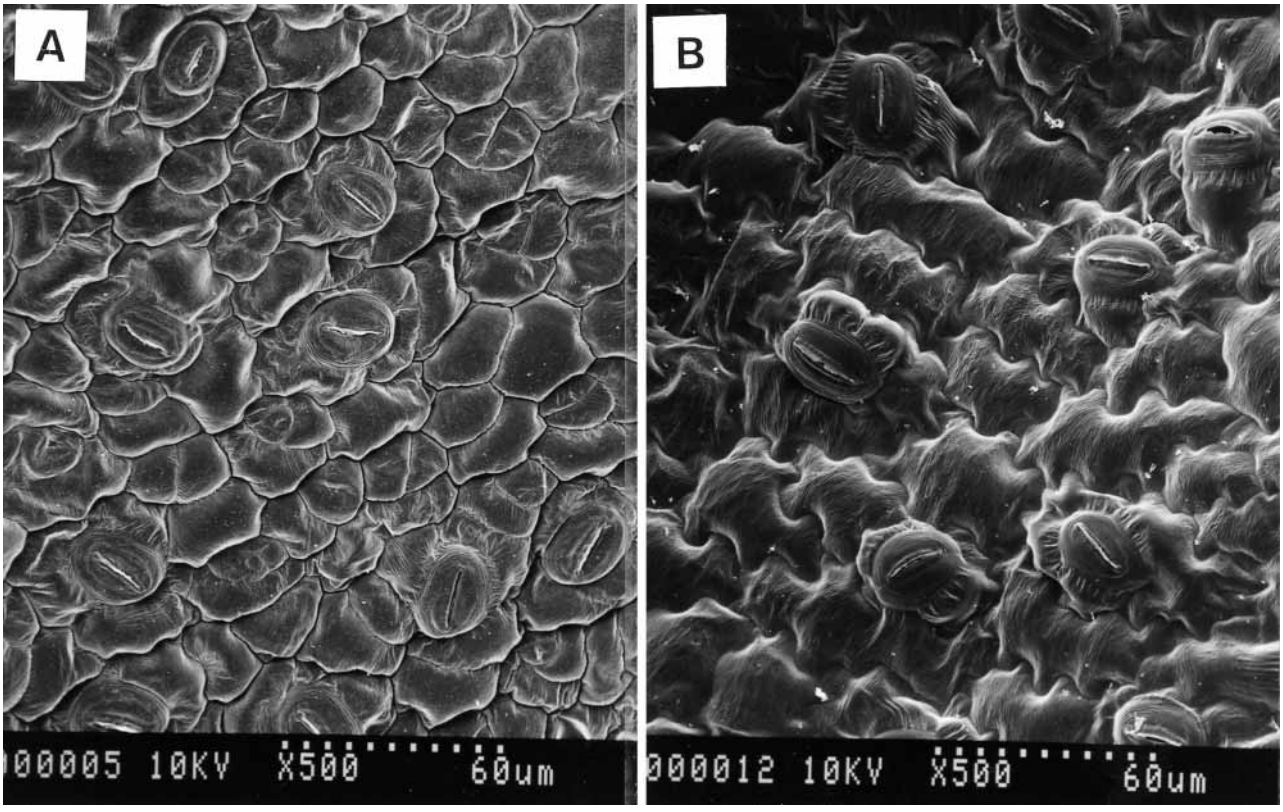

Fig. 3. Scanning electron micrographs of leaf adaxial epidermis (× 500). Terminal leaflet of leaf developed during heat stress while plant was supplied with 25 (Left) or 600 μ*M* Ca (Right). Leaves were mature at harvest and of the same position on the primary axis relative to the smallest leaf present at the initiation of heat stress.

Rudich 1985, Prange et al. 1990, Reynolds et al. 1990, Basu and Minhas 1991, Midmore and Prange 1992, Havaux 1994, Thornton et al. 1996) under heat stress have been reported. Through a combination of visual and quantitative data, we are the first to demonstrate that heat stress-induced reduction in leaf area may result primarily from impaired cell expansion (Table 1). Preliminary observations also suggest that disruptions in leaf growth are not likely to result from physical impedance of nutrient and water flow through the primary shoot axis since lignified vascular elements in leaves and stems of plants simultaneously exposed to high temperature and low Ca^{2+} treatment appeared normal (data not shown). Like other aspects of growth, we also provide initial evidence that impairment of cell expansion under heat stress can be partially overcome at specific, elevated levels of Ca^{2+} in the root zone (Table 1).

Microscopic examination of the first leaf visually observed to develop entirely under heat stress proved useful in explaining Ca^{2+} effects described here. Visual observations coupled with measurements of cell size and number (Table 1) suggest that reduced cell expansion led to the reduced leaf area in low Ca-treated and heat-stressed plants. Normal adaxial epidermal ground cells described by McCauley and Evert (1988b) contained scalloped margins. The rounded cell shape in low Ca-treated and heat-stressed plants of our study (Fig. 3) suggests that these cells had not fully expanded but that higher root zone Ca^{2+} levels allowed greater leaf epidermal cell expansion. Also, stomatal index was unaffected by Ca^{2+} level in this study (Table 1), suggesting that differentiation of epidermal cells into ground cells or stomatal complexes was Ca-independent. Together, these findings are especially relevant in light of recent evidence that low Ca^{2+} levels may induce polygalacturonase-mediated breakdown of cell wall polysaccharides and the release of biologically active cell wall fragments in potato leaves, even under normal temperatures (Seling et al. 2000). Data reported here and those of Seling et al. (2000) would suggest that root zone Ca^{2+} levels required to maintain normal cell structure and cell-to-plant development are greater under heat stress than normal temperatures. Possible disruption of fundamental, primary events in leaf development, however, makes direct interpretation of treatment effects on stomatal index difficult here. Leaf growth rates depend on massive and irreversible expansion of young cells produced by cell division in meristematic tissues (Snir and Neumann 1997). Nutrient supply could affect leaf growth by altering cell production and/or expansion (Snir and Neumann 1997). Snir and Neumann grew maize plants in incomplete nutrient solutions and reported that reductions in leaf elongation were associated with reductions in the final length of mature cells, rather than reduced cell division. The authors cautioned, however, that growth responses of dicot and monocot leaves to nutrient deficiency differ (Radin 1983, Snir and Neumann 1997). Wieneke (1995) recorded a 90% reduction of NO_3^- influx in squash root cells within 48 h of Ca^{2+} deprivation. Roots also displayed less turgor and growth and did not recover after transfer to Ca^{2+}-containing medium within 48 h of Ca^{2+} deprivation (Wieneke 1995). Interestingly, 10 μM Ca^{2+} was sufficient to mitigate the deficiency effect on NO_3^- influx (Wieneke 1995). Here we emphasize that overall leaf development was impacted by high temperature and Ca^{2+} treatment and that additional data are required to fully describe the influence of Ca^{2+} level on cell division and expansion under heat stress. Identifying the relative effects of temperature and Ca^{2+} treatment on epidermal morphology is also necessary and would provide further insight into data of Table 1. These data suggest that epidermal cell expansion in specific terminal leaflets exposed to heat stress was closely associated with root zone Ca^{2+} levels, being markedly inhibited at low Ca^{2+} levels (Table 1). These results provide insight into the mechanism by which root zone Ca^{2+} may modulate plant response to heat stress.

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