POTENTIAL FOR IMPROVING FREEZING STRESS TOLERANCE OF WILD POTATO GERMPLASM BY SUPPLEMENTAL CALCIUM FERTILIZATION

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Abstract

The main objective of the present study was to investigate the influence of supplemental calcium fertilization on the levels of freezing tolerance in different wild potato species. For this purpose, the freezing tolerance of a broad spectrum of the *Solanum* taxa was evaluated with and without supplemental calcium fertilization. Previous studies have shown that there is a large variation in the calcium accumulation capabilities among species and among various accessions within species. While this study confirms such variation, no direct relationship between the leaf calcium content and the improvement in freezing tolerance was found. Nevertheless, overall 45% of the species accessions had significant higher leaf calcium content and 52% of the species accessions showed a significant drop in the frost score average at the $p<0.05$ level. In terms of this experiment, a drop in the frost scores meant an improvement to withstand cold. One third of the frost tolerant accessions and 41% of the frost sensitive accessions showed both, a significant increase in leaf calcium content and at the same time a significant lower average frost score. The different response observed among the accessions may suggest that even though most of the accessions are able to accumulate calcium, only some of them may have the ability to benefit from the supplemental calcium. Future breeding schemes may be able to use this information for selecting clones that would respond positively to calcium fertilization in terms of frost survival. Results of this study also suggest that calcium fertilization may be used as a rather inexpensive cultural practice for protecting potatoes from frost damage. The results of the present study also suggest that soil calcium levels should be taken into consideration when freezing tolerance of potato germplasm is being evaluated.

Compendio

El objetivo principal de este trabajo fue investigar si la fertilización con calcio puede incrementar los niveles de tolerancia a heladas en diferentes especies silvestres de papa. Para esto, la tolerancia a heladas fue evaluada en varias especies del género *Solanum* con y sin calcio como suplemento. Estudios anteriores han demostrado una gran variabilidad inter e intra-especifica (difer-

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encia entre especies y accesiones de una especie) en la capacidad de acumular calcio. Además de confirmar dicha variabilidad, nuestros resultados también demostraron que no existe una relación directa entre la concentración de calcio en las hojas y la resistencia a heladas. Sin embargo, 45% de las accesiones aumentaron significativamente la concentración de calcio en las hojas y 52% de las accesiones aumentaron significativamente su tolerancia a heladas ($p<0.05$). Un tercio de las accesiones tolerantes a heladas y 41% de las accesiones susceptibles a heladas exhibieron un aumento significativo en el contenido de calcio en las hojas y mayor tolerancia a heladas. Esta diferencia en la respuesta al daño por heladas sugiere que a pesar que la mayoria de accesiones son capaces de acumular calcio, sólo algunas tienen la habilidad de utilizar calcio para mejorar su tolerancia a bajas temperaturas. Futuros programas de mejoramiento pueden utilizar esta información para la selección de clones que respondan positivamente a la fertilización con calcio en términos de sobrevivencia a bajas temperaturas. Adicionalmente, nuestros resultados sugieren que la fertilización con calcio puede ser un factor importante y económico en la protección del cultivo de papa contra el daño causado por heladas. Los resultados de este trabajo también sugieren la consideración de los niveles de calcio en el suelo durante la evaluación del germoplasma de papa con resistencia a heladas.

Introduction

Species of wild potato (*Solanum* sect. Petota) are found in extensive areas of South and North America, growing over an unusually wide range of altitudes, from sea-level up to nearly 5000 m. The greater majority, however, grow in the cool climates of the highlands and inter-Andean valleys of South America between 2000 and 4000 m (29). Since the range of climatic adaptation is much wider in wild potato species than in the most common commercially cultivated potato (*Solanum tuberosum* L.), they are known to possess different levels of frost tolerance and/or cold acclimation capacity (12, 13, 14).

Solanum tuberosum possesses little or no frost tolerance (14). In this species, freezing damage to foliage results in extensive reductions in tuber yield and limits its cultivation. In the U.S., one-sixth of the cultivated area is subjected to cold limitations (6). In many cases it is not possible to adjust planting times to avoid damage since frosts occur sporadically or throughout the growing season (27). There is therefore a need for potato cultivars with some levels of frost tolerance. The development of frost tolerant clones could greatly expand potato production to areas which are currently marginal due to low temperature. Additionally, potato productivity also could be increased in the presently cultivated areas by simply extending the growing season (13).

Frost tolerance and cold acclimation are separate heritable traits, suggesting that progress in the improvement of freezing tolerance can be made by individually selecting for these two components of freezing tolerance (23, 30). Evidence suggests that Ca^{2+} is an important factor in the maintenance of membrane integrity and membrane transport functions (7, 9, 10, 25). Freezing injury results in increased leakage of ions and organic solutes and in water soaking of the plant tissue (18, 19, 22, 32). These symptoms suggest that the cell membrane is a site of freezing injury (21). Although the major ion that leaks out of the injured cells is K^+ (18, 20, 32), there is also a small but significant loss of cellular/membrane Ca^{2+} during early stages of freeze-thaw stress (1, 2, 3, 18). The progress of injury can be halted by bathing/washing the freeze-thaw injured tissue with $CaCl_2$ solution (1, 18). There is also some evidence that cellular calcium may play an important role in the recovery of freezing injury, which is associated with the activity of plasma membrane H^+-ATPase (4, 24). These studies suggest that maintenance of cellular/membrane calcium may be important for frost survival.

The main objective of our study was to find out if supplemental calcium fertilization could increase the levels of freezing tolerance in a broad spectrum of the tuber-bearing *Solanum* species. The exposure to frost in the natural environment represents an ultimate test for potato frost tolerance (13). Therefore, the present studies were carried out under field conditions.

Materials and Methods

Plant Material

A total of 88 accessions, representing eleven frost-tolerant and ten frost-sensitive species, were chosen based on our previous frost scores (33). Seeds were germinated in 10 cm clay pots filled with a peat-vermiculite mixture (Jiffy Mix, JPA, East Chicago, IL). When the seedlings were about 5 to 8 cm tall, they were transferred to 6 cm pots with the same potting mix. After three weeks, the plants were transplanted to the field in 12 rows (122 cm between the rows) at the Peninsular Agricultural Research Station, Sturgeon Bay, Wisconsin. Each plot consisted of four plants. A distance of 60 cm was left between plots and 30 cm between the plants within a plot.

Calcium Applications

The two treatments used were with and without supplemental calcium. Calcium nitrate was used as the calcium source. The supplemental calcium treatment consisted of 167 kg ha^{-1} of calcium, divided into three applications of 55.6 kg ha^{-1} each on August 6th, 31st and September 17th, 1993. The treatment without extra calcium received an equivalent amount of nitrogen in the form of ammonium nitrate on the same three dates. Both fertilizers were dissolved in two liters of water and applied on the top of the plants with a watering can. After each application, a light irrigation was administered to wash the solutions off the foliage and into the soil.

Calcium Analysis Procedure

A composite sample, consisting of the youngest fully developed leaves, was taken from each plot before the first frost (October 6th). Each sample was processed according to the method of Kratzke and Palta (11). The samples were oven-dried at 70 C for 4 days, then ground using a coffee grinder (Fast-Touch Coffee-Mill #203, Krups, Denver, Colorado, USA). Duplicates of each 50 mg sample were weighed into 10 ml beakers, ashed at 450 C for 6 hours and digested in 5 ml of 2N HCl. The beaker content was then filtered through Whatman 540 paper followed by several rinses with distilled water. The volume of the filtrate was brought up to 50 ml with distilled water containing 10 ml of 0.2N HCl - 10,000 ppm $LaCl_3$ (Lanthanum chloride) solution. Measurements of the calcium concentration were made using an atomic absorption spectrophotometer (AA-20, Varian Associates, Inc. USA).

Temperature Records

Temperature was monitored throughout the field at different levels above the ground and in different locations in the canopy by using a data logger with 20 thermocouples. The thermocouples were placed into leaves, stems, axillary branches and berries, and in air shaded at 50 cm and 90 cm above the ground. Temperatures were recorded every 30 seconds.

Frost Scores

Frost scoring was done at the end of the season, when natural frosts appeared to best differentiate the species. The first frost score was taken October 3rd after an early mild frost which was not recorded. A second mild frost was recorded on October 6th and the first hard frost was recorded on October 11th. Frost scores for each plot were again taken on October 8th and 13th by using a visual scale from 1 to 6, where: 1 = no damage, 2 = slight bronzing, 3 = some top leaflets killed, 4 = all top leaves killed, 5= all leaves and petioles killed and 6 = leaves and stems (plant) killed. A similar frost injury scale used in our earlier studies was found to be satisfactory for rating potato foliage (33).

Statistical Design

The experiment was laid out in a split plot design with sub-sampling, having calcium as the main plot and the potato species accessions as the subplot. There were two replications, and two sub-samples were taken from each replication for calcium analysis. The data were statistically analyzed using the SAS computer program (28).

Results and Discussion

Frost Scores and Temperature Data

Freezing damage was quickly evident by wilting and blackening of the foliage. Previous experiments have shown that ratings of foliage injury were about the same whether the ratings were made on the afternoon following the frost or a few days later (27), therefore, the scores were taken one or two days after the frosts. Two mild frosts on October 2nd and 8th, and one hard frost on October 13th were detected. Temperatures were recorded for the frosts on October 8th and 13th with lowest temperatures of around - 2 C and - 4.5 C, respectively. Three frost scores were taken, October 3rd and 10th after each of the two mild frosts, and October 16th after the first hard frost. The averages from the three frost scores are presented in Tables 1 and 2.

TABLE 1.—*Leaf calcium concentration and frost scores of frost tolerant wild* Solanum *accessions with and without supplemental calcium.*

Solanum species	species accession	Frost score[a] (-) Ca	Frost score[a] (+) Ca	Ca(mg/g)[b] (-) Ca	Ca(mg/g)[b] (+) Ca
S. acaule	175396	2.2	1.5**	13.1	18.2**
	195160	1.5	1.5	9.4	10.1
	210029	1.5	1.7	15.7	16.9
	210033	1.5	1.5	10.2	14.7**
	320276	1.8	1.5**	11.3	15.1**
	472641	2.0	1.3**	12.4	11.1
S. boliviense	545853	4.2	4.5	20.3	23.8**
	545889	4.8	2.8**	21.3	28.2**
	545964	3.7	3.2**	21.4	16.3
	545966	4.3	3.3**	20.9	24.1*
S. brevidens	558175	4.2	3.7**	15.7	18.1*
	558242	3.7	3.7	9.7	10.7
	558283	4.3	3.3**	13.2	14.2
S. bukasovii	473452	3.7	2.2**	14.4	12.7
	473491	3.3	2.5**	22.3	24.3*
	473493	2.2	2.0*	19.2	22.5**
	473494	3.8	2.5**	15.2	13.0
S. canasense	246533	3.5	3.0**	16.6	19.0*
	310939	4.2	3.7**	20.5	21.1
	458375	3.3	3.7	18.4	14.1
	458377	4.0	3.7**	12.0	13.7
	473346	3.0	3.3	12.7	13.1
S. commersonii	472834	2.5	2.2**	17.5	19.8*
	472835	2.5	2.2**	25.0	22.1
	472836	3.8	2.2**	28.4	36.4**
S. demissum	161163	2.2	2.3	17.7	13.5
	161165	2.7	2.7	11.3	14.2*
	161167	2.2	2.5	13.2	16.9**
	218047	2.5	2.3*	17.1	25.3**
	225652	2.2	2.3	20.2	31.4**
S. megistacrolobum	498383	1.8	2.8	22.5	25.7**
	500031	3.2	2.3**	15.5	19.6**
	545999	3.2	2.2**	22.7	17.0
	546000	2.5	3.0	21.8	19.2
S. sanctae-rosae	275152	3.5	3.3*	24.9	21.7
	283089	4.3	4.5	21.9	27.9**
	473200	4.0	2.2**	22.4	18.4
	498391	2.7	2.0**	17.2	22.7**
	498393	5.3	3.5**	26.5	17.1
S. toralapanum	498144	2.8	3.2	25.7	25.2
	498145	3.0	2.5**	22.3	22.9
	545892	2.0	3.2	18.1	21.1*
	546009	4.8	3.7**	14.1	16.2*
	546015	4.0	2.2**	16.9	17.8

TABLE 1—*Continued.*

Solanum species	species accession	Frost score[a] (-) Ca	Frost score[a] (+) Ca	Ca(mg/g)[b] (-) Ca	Ca(mg/g)[b] (+) Ca
S. vernei	230468	3.5	2.5**	9.0	14.1**
	320332	3.7	3.0**	8.4	10.8*
	320333	4.0	4.0	12.3	11.3
	458369	3.7	3.0**	13.4	15.2
	458370	3.7	2.8**	11.1	10.4

[a]Leaf calcium concentrations are averages from two replications with two subsamples each.
[b]Frost scores are averages from three scores taken on October 3rd, 10th and 16th 1994 on the two replications. The frost scores are on a scale from 1 to 6, where 1 = no damage and 6 = killed (see materials and methods for complete scale).
*$P \leq 0.10$ (improvement in frost score/higher leaf calcium content after calcium aplications).
**$P \leq 0.05$ (improvement in frost score/higher leaf calcium content after calcium aplications).

Leaf Calcium Content and Species Frost Tolerance

The average leaf calcium content and frost scores of the species accessions are shown in Tables 1 and 2. The analyses based on the split plot design used for the experiment resulted in highly significant differences among the accessions for both their ability to accumulate calcium and their frost tolerance (Table 3). The significant difference in the ability to take up and/or accumulate calcium was expected among the accessions of the various species. This is in agreement with the recent results of Bamberg *et.al.* (5), where a large variation in tuber calcium concentration was found in different *Solanum* species.

Since we deliberately selected potato species with various frost tolerance levels for this experiment, the substantial difference in frost tolerance was expected among the different species accessions. These results are also in agreement with previous reports (13, 33). The commonly cultivated species *S. tuberosum* was not included in our experiment. However, an adjacent plot in the same field confirmed its high sensitivity to freezing stress.

In general, a drop in frost score (an increase in frost survival) accompanied the application of extra calcium (Table 1). A highly significant interaction between supplemental calcium and accessions was found (Table 3). This was true when either the frost tolerance or the leaf calcium content were considered as independent variables. Overall, 45% of the accessions had significant higher leaf calcium content and 52% of the accessions showed a significant improvement in frost survival at the $p<0.05$ level (Table 1). A closer look at the data shows that 33% of the frost tolerant accessions and 41% of the frost sensitive accessions showed both a significant increase in leaf calcium content and a significant increase in frost survival. This tendency of the accessions to have higher leaf calcium content and improvement in frost survival (lower frost

TABLE 2—*Leaf calcium concentration and frost scores of frost sensitive wild* Solanum *accessions with and without supplemental calcium.*

Solanum species	species accession	Frost score[a] (-) Ca	Frost score[a] (+) Ca	Ca(mg/g)[b] (-) Ca	Ca(mg/g)[b] (+) Ca
S. berthaultii	310927	5.0	5.0	25.1	37.7**
	473337	4.5	4.3*	33.4	31.0
	498100	5.0	4.3**	25.4	32.6**
	545851	4.8	4.8	24.8	29.8**
	558033	5.0	4.5**	35.5	34.1
S. bulbocastanum	243511	5.7	5.7	34.2	33.5
	253210	5.5	5.0**	20.3	23.9**
	275189	5.7	5.0**	22.4	22.0
	275199	5.7	4.3**	27.0	23.5
S. cardiophyllum	184766	5.8	5.7*	19.8	22.0*
	251725	6.0	6.0	21.4	29.4**
	279303	5.7	5.3**	29.1	38.2**
	283062	6.0	5.7**	16.3	31.4**
S. chacoense	133123	4.3	4.5	29.6	38.8**
	133618	5.0	4.0**	35.5	30.8
	275142	5.0	5.0	34.1	28.9
	472825	4.3	4.3	23.3	28.0**
S. fendleri	275156	5.7	5.0**	14.9	20.8**
	275161	6.0	5.8*	20.1	30.7**
	283102	6.0	5.2**	21.6	30.1**
	458420	6.0	5.8*	17.8	18.5
	558395	6.0	5.0**	19.2	29.4**
S. microdontum	473167	4.8	5.0	20.9	17.1
	498124	5.0	5.0	22.3	33.0**
	498125	4.3	4.3	32.8	31.8
S. phureja	225678	5.0	4.8*	17.9	22.9**
	225683	5.7	4.8**	12.1	22.4**
	273453	5.7	4.8**	17.9	21.4**
	320376	4.3	4.3	17.7	28.9**
	320377	5.5	4.7**	11.4	20.7**
S. polyadenium	558485	6.0	5.8*	15.6	17.7*
S. pinnatisectum	184764	4.8	6.0	10.9	16.4**
	190115	4.8	4.7*	11.7	15.1**
	275230	4.7	4.3**	13.4	14.0
	275231	4.8	5.5	7.5	18.0**
	275232	5.7	6.0	15.1	21.8**
S. verrucosum	275258	5.7	5.7	12.4	19.1**
	558457	5.0	4.7**	20.2	23.3*
	558488	6.0	5.5**	16.1	14.8

[a] Leaf calcium concentrations are averages from two replications with two subsamples each.

[b] Frost scores are averages from three scores taken on October 3rd, 10th and 16th 1994 on the two replications. The frost scores are on a scale from 1 to 6, where 1 = no damage and 6 = killed (see materials and methods for complete scale).

*P ≤ 0.10 (improvement in frost score/higher leaf calcium content after calcium aplications).

**P ≤ 0.05 (improvement in frost score/higher leaf calcium content after calcium aplications).

TABLE 3—*ANOVA tables for both frost scores and leaf calcium content.*

Dependant Variable: Frost tolerance

Source of variation	df	Pr > F
Accession	87	0.0001
Calcium treatment	1	0.0001
Calcium treatment x Accession	87	0.0001

R-square	C.V.	Root MSE
0.99	3.60	0.14

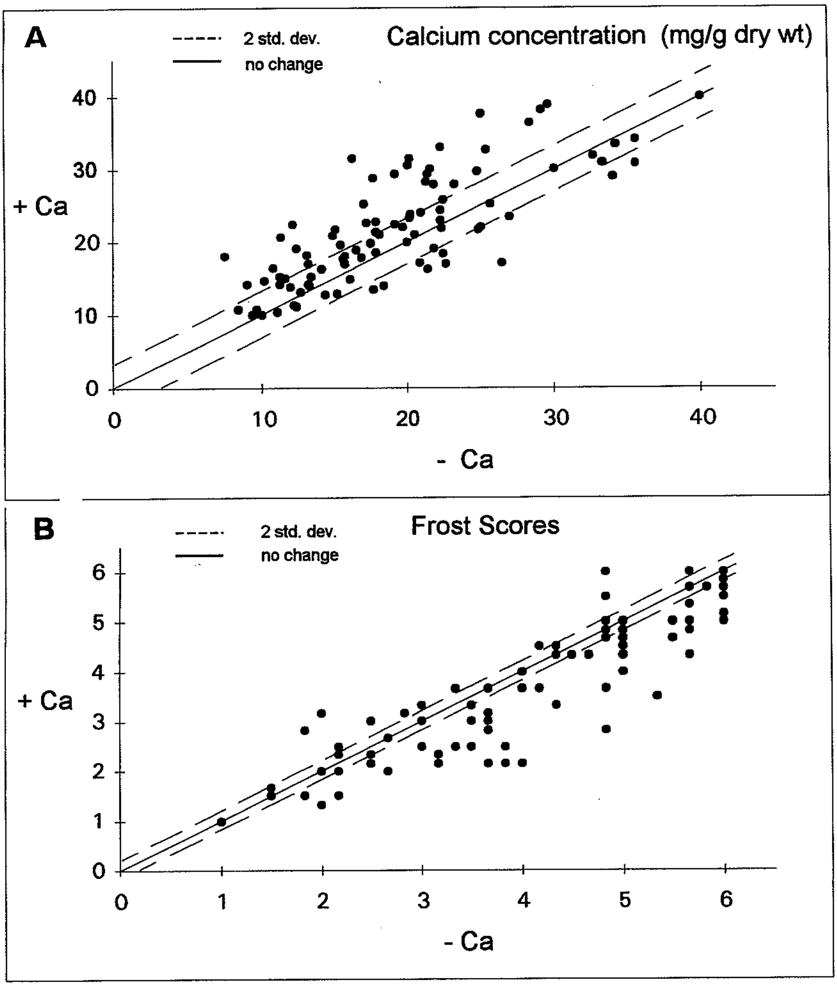
Dependant Variable: Leaf Calcium Concentration

Source of variation	df	Pr > F
Accession	87	0.0001
Calcium treatment	1	0.0001
Calcium treatment x Accession	87	0.0001

R-square	C.V.	Root MSE
0.96	11.11	2.23

scores) after supplemental calcium application is demonstrated in Figure 1A and 1B, respectively. From the 49 frost tolerant accessions, 24 showed a significantly higher leaf calcium content, 32 accessions significantly improved their frost survival and 16 accessions were significant for both after supplemental calcium application. From the 39 frost sensitive accessions, 27 showed a significantly higher leaf calcium content, 24 accessions significantly improved their frost survival and 16 accessions were significant for both after calcium fertilization. Not all of the accessions that showed a significant increase of the calcium content in the leaf tissue showed also a significant improvement in frost survival. The different response observed among the accessions suggests that even though some accessions are able to accumulate calcium, they may lack the ability to benefit from the supplemental calcium.

Although a number of accessions responded positively to calcium fertilization in terms of improvement of frost survival, the data from this trial failed to show a systematic or linear relationship between the specific leaf calcium content and the frost tolerance (*e.g.* higher leaf calcium content = more freezing tolerance) (Fig. 2 A and B). This is expected since accumulation of calcium in the leaves depends in part upon the transpiration rate and ability of the root system to efficiently supply water and calcium. Variations among species and accessions within a species for these parameters are expected. The new and interesting finding is the ability of many accessions to benefit from

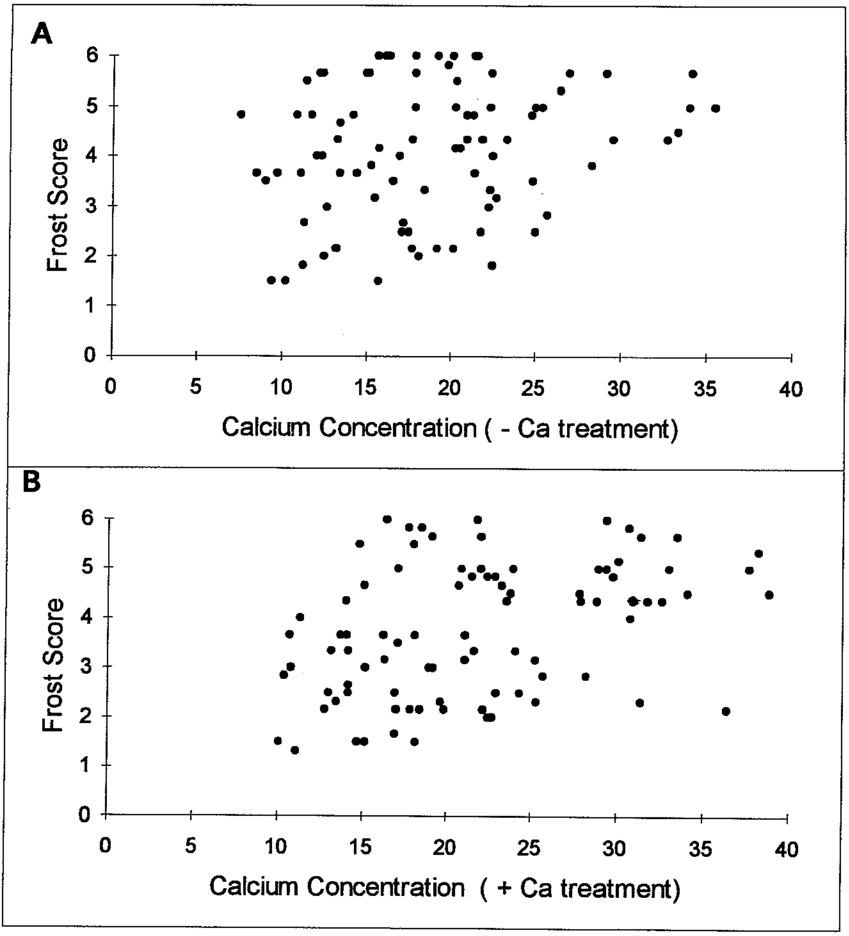


[a] Leaf calcium concentrations are averages from two replications with two subsamples each.

[b] Frost scores are averages from three scores taken on October 3rd, 10th and 16th 1994 on the two replications. The frost scores are on a scale from 1 to 6, where 1 = no damage and 6 = killed (see materials and methods for complete scale).

FIG. 1. Leaf calcium content[a] (A) and frost scores[b] (B) of the *Solanum* accessions with (+ Ca) and without (- Ca) supplemental calcium.

supplemental calcium in terms of improvement in frost tolerance. Furthermore, both sensitive species (*i.e. S. bulbocastanum, S. fendleri, S. phureja,* etc.) and very hardy species (*i.e. S. boliviense, S. bukasovii, S. commersonii, S. vernei,* etc.) responded positively to supplemental calcium. This result show a wide promise for improvement of frost tolerance by calcium.



[a] Leaf calcium concentrations (mg/g dry wt) are averages from two replications with two subsamples each.

[b] Frost scores are averages from three scores taken on October 3rd, 10th and 16th 1994 on the two replications. The frost scores are on a scale from 1 to 6, where 1 = no damage and 6 = killed (see materials and methods for complete scale).

FIG. 2. Relationship between leaf calcium content[a] and frost scores[b] of the *Solanum* accessions with (+ Ca) and without (- Ca) supplemental calcium.

Several reports have suggested the general protective effect of Ca^{2+} on many cellular processes during stress (7, 8, 15, 16, 17). Calcium has been recognized as an important factor in the maintenance of membrane integrity and as a regulator of ion transport (7, 9, 10, 25, 26). Arora and Palta (3) demonstrated that a loss of membrane-associated Ca^{2+} from freeze-thaw stressed cells is accompanied by leakage of ions and water soaking of the tissue. Their studies also showed that extracellular calcium is able to prevent irreversible symptoms of freezing injury in onion bulb scale cells (1). Therefore, it may be possible that some accessions have a mechanism by which supplemental calcium will result in a protective effect against freezing stress.

Freezing tolerance is known to be a genetically complex trait (31). Several physiological factors are recognized to be associated with this trait (23, 24). Our results indicate that calcium could be one of the several factors involved in freezing tolerance. Thus, soil calcium levels should be taken into consideration while evaluating the freezing tolerance of potato germplasm. Furthermore, it may be appropriate to screen potato accessions with the ability to improve their freezing tolerance in the presence of supplemental calcium. The identification of factors that contribute to such ability in this wild germplasm could be then transferred to cultivars which would allow us to give some additional frost protection to the crop by a relatively simple and inexpensive application of calcium. Interestingly, we found that *S. phureja,* a cultivated species, was able to benefit from the supplemental calcium fertilization. This could be incorporated into a breeding program aimed at improving the freezing tolerance of potatoes. However, further investigation is needed to characterize germplasm with respect to this phenomenon and elucidate its genetic and physiological basis.

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Literature Cited

1. Arora, R. and J.P. Palta. 1986. Protoplasmic swelling as a symptom of freezing injury in onion bulb cells. Plant Physiol 82:625-629.
2. Arora, R. and J.P. Palta. 1988. *In vivo* perturbation of membrane-associated calcium by freeze-thaw stress in onion bulb cells. Plant Physiol 87:622-628.
3. Arora, R. and J.P. Palta. 1989. Perturbation of membrane calcium as a molecular mechanism of freezing injury. *In:* Environmental Stress in Plants. Cherry J.H. (ed.) Springer-Verlag. Berlin pp. 281-290.
4. Arora, R. and J.P. Palta. 1991. A loss in the plasma membrane ATPase activity and its recovery coincides with incipient freeze-thaw injury and postthaw recovery in onion bulb scale tissue. Plant Physiol 95:846-852.
5. Bamberg, J.B., J.P. Palta, L.A. Peterson, M. Martin, and A.R. Krueger. 1993. Screening tuber-bearing *Solanum* (potato) germplasm for efficient accumulation of tuber calcium. Am Potato J 70:219-226.
6. Boyer, J.S. 1982. Plant productivity and environment. Science 18:443-448.
7. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant Physiol 36:437-444.
8. Hanson, J.B. 1960. Impairment of respiration, ion accumulation and retention in root tissue treated with ribonuclease and EDTA. Plant Physiol 35:372-379.

9. Hanson, J.B. 1984. The function of calcium in plant nutrition. *In:* Advances in plant nutrition, Vol. I. Tinker, P.B., Läuchli A. (eds.) Praeger, NY, pp. 149-208.
10. Hepler, P.K. and R.O. Wayne. 1985. Calcium and plant development. Ann Rev Plant Physiol 36:397- 439.
11. Kratzke, M.G. and J.P. Palta. 1986. Calcium uptake by potato tubers: Role of the basal roots. HortScience 21:1022-1024.
12. Li, P.H. 1977. Frost killing temperatures of 60 tuber-bearing *Solanum* species. Am Potato J 54:452-456.
13. Li, P.H. and J.P. Palta. 1978. Frost hardening and freezing stress in tuber-bearing *Solanum* species. *In:* Plant Cold Hardiness and Freezing Stress. Sakai, H.A. (ed.) Academic Press. Inc. NY, USA, pp. 49-71.
14. Li, P.H., J.P. Palta, and H.H. Chen. 1979. Freezing stress in potato. *In:* Low Temperature Stress in Crop Plants. Role of cell membranes. J. Lyons, D. Graham and J. Raison (eds.) Academic Press. Inc. NY, USA, pp. 291-303.
15. Matsubra, H. 1976. Some properties of thermolysin. Molecular mechanism of temperature adaptation. Am Assoc Adv Sci Publ 84:283-294.
16. Overstreet, R., L. Jacobson, and D. Hamdley. 1952. The effect of calcium on the absorption of potassium by barley roots. Plant Physiol 27:583-590.
17. Palta, J.P. 1996. Role of calcium in plant responses to stresses: linking basic research to the solution of practical problems. HortScience, Vol. 31(1): 51-57.
18. Palta, J.P., J. Levitt, and E.J. Stadelmann. 1977. Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. Plant Physiol 60:393-397.
19. Palta, J.P., J. Levitt, and E.J. Stadelmann. 1977. Freezing injury in onion bulb cells. II. Post thawing injury or recovery. Plant Physiol 60:398-401.
20. Palta, J.P., J. Levitt, and E.J. Stadelmann. 1977. Freezing tolerance of onion bulbs and significance of freeze-induced tissue infiltration. Cryobiology 14:614-619.
21. Palta, J.P. and P.H. Li. 1978. Cell membrane properties in relation to freezing injury. *In:* Plant cold hardiness and freezing stress. Li, P.H. and Sakai, A. (eds.) Academic Press, NY, USA, Vol 1, pp. 93-115.
22. Palta, J.P. and P.H. Li. 1980. Alterations in membrane transport properties by freezing injury in herbaceous plants. Evidence against rupture theory. Physiol Plant 50:169-175.
23. Palta, J.P. and G. Simon. 1992. Breeding potential for improvement of freezing stress resistance: genetic separation of freezing tolerance, freezing avoidance, and capacity to cold acclimate. *In:* Advances in Plant Cold Hardiness. Li, P.H. and Christersson, L. (eds.), CRC Press, Inc. Boca Raton, FL, USA, pp. 299-310.
24. Palta J.P and L.S. Weiss. 1992. Ice formation and freezing injury: An overview on the survival mechanisms and molecular aspects of injury and cold acclimation in herbaceous plants. *In:* Advances in plant cold hardiness. Li, P.H. and Christersson, L. (eds.) CRC Press Inc. Boca Raton, FL, USA, pp. 143-176.
25. Poovaiah, B.W. and A.C. Leopold. 1976. Effects of inorganic salts on tissue permeability. Plant Physiol 58:182-185.
26. Poovaiah, B.W., A.S.N. Reddy, and J.J. McFadden. 1987. Calcium messenger system: role of protein phosphorylation and inositol bisphospholipids. Physiol Plant 69:569-573.
27. Richardson, D.G. and N. Estrada. 1971. Evaluation of frost resistant tuber-bearing *Solanum* hybrids. Am Potato J 48:339-343.
28. SAS Institute, Inc. 1989. SAS/STAT User's Guide. Version 6, 4th ed. SAS-Institute, Inc. Cary, N. Carolina 2:846.

29. Smillie, R.M., S.E. Hetherington, C. Ochoa, and P. Malagamba. 1983. Tolerances of wild potato species from different altitudes to cold and heat. Planta 159:112-118.
30. Stone, J.M., J.P. Palta, J.B. Bamberg, L.S. Weiss, and J.F. Harbage. 1993. Inheritance of freezing resistance in tuber-bearing *Solanum* species: Evidence for independent genetic control of nonacclimated freezing tolerance and acclimation capacity. Proc Natl Acad Sci 90:7869-7873.
31. Stushnoff, C., B. Fowler, and A. Bruelé-Babel. 1984. Breeding and selection for resistance to low temperature. *In:* Crop Breeding, a Contemporary Basis. Vose, P.B. and Blixt, S.G. (eds.) Pergamon Press, 1st Edition, Oxford pp. 115-136.
32. Sukumaran, N.P. and C.J. Weiser. 1972. Freezing injury in potato leaves. Plant Physiol 50:564-567.
33. Vega, S.E. and J.B. Bamberg. 1995. Screening the US potato collection for frost hardiness. Am Potato J 72:13-21.