

RELATIONSHIP BETWEEN HEAT AND FROST RESISTANCE OF TUBER-BEARING SOLANUM SPECIES: EFFECT OF COLD ACCLIMATION ON HEAT RESISTANCE¹

J. P. PALTA,* H. H. CHEN,† AND P. H. LI†

* Department of Botany, University of Iowa, Iowa City, Iowa 52242; and † Laboratory of Plant Hardiness, Department of Horticultural Science and Landscape Architecture, University of Minnesota, Saint Paul, Minnesota 55108

A possible relationship between heat and freezing-stress resistance was investigated in four potato species. These species differed in degree of freezing-stress resistance and ability to acclimate to cold. The 2, 3, 5-triphenyl tetrazolium chloride reduction and the conductivity test were used to evaluate the relative heat and freezing-stress resistance of excised leaflets. Data from both tests failed to show a systematic relationship between heat and freezing-stress resistance in the four potato species. The cultivated species, *Solanum tuberosum*, had the lowest level of freezing-stress resistance and one of the highest levels of heat-stress resistance. One noncultivated accession had a high degree of frost and heat resistance, whereas the other three accessions had a high degree of frost but poor heat resistance. Fifteen days at 5/2 C day/night did not increase heat- and freezing-stress resistance of *S. tuberosum* but increased both heat- and freezing-stress resistance in *S. commersonii* and other species capable of cold acclimation. These results show that (1) resistance to heat and freezing stress is not always mutually exclusive; (2) if a plant is capable of increasing freezing-stress resistance during cold acclimation, it also seems to have the ability to increase heat-stress resistance; and (3) the enzymes that participate in oxidation-reduction reactions and active transport of ions are sensitive to heat injury.

Introduction

Many plants develop freezing-stress resistance in response to low temperatures in autumn, reach a maximum in winter, lose it rapidly in spring, and reach a minimum in summer (LEVITT 1972). Also, most plants reach a maximum of heat-stress resistance in summer (LEVITT 1972). Tropical plants well adapted to high temperature are generally sensitive to chilling and freezing. From these general observations, resistance to heat and freezing-stress seems to be mutually exclusive; this is supported by FELDMAN and KAMENTZEVA (1963) and LEVITT (1972). ALEXANDROV (1964), however, reported that some plants in autumn developed freezing and heat resistance at the same time.

We found that several tuber-bearing *Solanum* species vary considerably in their frost resistance (LI 1977; PALTA and LI 1979) and in their ability to acclimate to cold (CHEN and LI 1976; LI, PALTA, and CHEN 1979). In the present study, we investigated the ability of some potato species to resist freezing stress and heat stress simultaneously. We examined the relationship between heat and frost resistance of related potato species and studied the effect of cold acclimation on heat resistance.

Material and methods

PLANTS AND GROWTH CONDITIONS.—Four tuber-bearing *Solanum* species were studied: *S. acule*, *S.*

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Address for correspondence and reprints: J. P. PALTA, Department of Botany, University of Iowa, Iowa City, Iowa 52242.

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multidissectum, and *S. commersonii* were propagated from seeds; *S. tuberosum* 'Red Pontiac' was propagated from tubers. Seeds were planted in 15-cm-diameter pots filled with a mixture of two parts loam soil, one part sand, and one part peat. Plants were raised in growth chambers with a 14-h photoperiod, 450 $\mu\text{E s}^{-1} \text{m}^{-2}$ radiation, and 20/15 C, day/night temperature regime. After emergence, plants were grown for about 2 mo before experiments began. Experiments were repeated at least three times.

HEAT STRESS.—Leaflets from the third or the fourth leaf from the top were excised, rinsed in deionized/distilled water, and placed at the bottom of 15-cm test tubes. The lower half of the test tube was lined with moist tissue paper to ensure high humidity in the tube. Tubes were stoppered with rubber stoppers. A thermometer, inserted through a hole in the center of the stopper, monitored the air temperature in the tube next to the leaflet. Constant temperature inside the test tubes was maintained by immersion in a water bath maintained at 45 or 50 C. Heat stress was varied by keeping the tubes at one temperature for different periods of time. After a certain time at the desired temperature, the tubes were removed from the bath and placed at room temperature for 1 h before viability of the tissue was evaluated.

FREEZING STRESS.—Test tubes containing excised leaflets were cooled at a rate of 1 C/h in a cooling bath (SUKUMARAN and WEISER 1972). The leaflets were inoculated with crushed ice at -1 C to initiate ice formation. At the desired freezing temperature (0.5, 1, or 2 C intervals), the samples were removed from the cooling bath and thawed at about 0 C over ice overnight before viability of the tissue was evaluated.

VIABILITY TESTS.—Two separate tests on the same leaflet were made: (1) the capability of tissue to re-

duce 2, 3, 5-triphenyl tetrazolium chloride (TTC) and (2) the leakage of ions from the tissue. At least five replicates were made for the TTC test and two replicates for the conductivity test. In each replication a separate leaflet was used.

1. TTC test: Except for some details, procedures were similar to those of TOWILL and MAZUR (1974). After heat or freezing stress, two disks 7 mm in diameter were cut from the central portion of each leaflet with a cork borer, transferred to a test tube containing 2.5 ml of 0.08% TTC solution in 0.05 M phosphate buffer, pH 7.4, vacuum infiltrated for 5 min, and incubated for 18–20 h in the dark. The disks were removed, rinsed with distilled water, and the reduced TTC extracted with 3 ml of 95% ethanol, which was boiled until the solvent was completely evaporated. After cooling, the residue was redissolved in 3 ml of 95% ethanol, and TTC reduction was estimated by measuring the absorbance at 485 nm, using a double-beam spectrophotometer. An ethanol extract from the nonstressed leaflets without TTC incubation was used as reference for calibrating the spectrophotometer. Tissue injury was expressed as the percentage of decrease in TTC reduction compared with nonstressed controls.

2. Conductivity test: The procedure for this test was similar to that of PALTA, LEVITT, and STADELMANN (1977a). The leaflet remaining after the disks were removed for the TTC test was used for the conductivity test. Leaf samples in plastic vials were immersed in 10 ml of deionized/distilled water, vacuum infiltrated, and shaken for 1 h to diffuse ions into the distilled water. Electrical conductance was measured in the vial with a standard conductivity bridge (R_1). The samples were quickly frozen to -40 C, warmed, and shaken for 1 h; the conductivity of the solution was read (R_2). The ratio of the two readings, R_1/R_2 , was used to measure relative injury.

VISUAL OBSERVATIONS.—After exposure to stress and just before the viability tests, samples were

observed for infiltration (darkening and soaking of the tissue) and for loss of turgor.

COLD ACCLIMATION.—Some plants grown for 2 mo in pots were transferred from 20/15 C day/night regime to 5/2 C day/night regime. The photoperiod and radiation level were the same under both regimes. After 15 days at low temperatures, heat-stress and freezing-stress resistances were measured.

Results

VIABILITY TESTS FOR HEAT- AND FREEZING-STRESS RESISTANCES.—Preliminary experiments showed that irreversible damage (inability to recover turgor loss and tissue soaking) coincided very closely with the 50% loss of both the total ions and TTC reduction. Both TTC and the conductivity tests assessed relative freezing and heat stress for all of the potato species (table 1). In general, the results from the two viability tests agreed. The freezing temperatures that coincided with a 50% leakage of solutes (50% conductivity) and a 50% loss in TTC reduction were -12.2 and -12.3 C for acclimated *Solanum commersonii* 'Oka 5040' and -4.2 and -4.7 C for nonacclimated *S. commersonii* (fig. 1). The standard deviation for the replicate means varied from $\pm 2\%$ to $\pm 12\%$ for the TTC test and $\pm 1\%$ to $\pm 10\%$ for the conductivity tests (figs. 1–3). The standard deviation, averaged from several experiments (figs. 1–3), was 3.5% for the conductivity test and 4.3% for the TTC test.

HEAT- AND FREEZING-STRESS RESISTANCES.—Relative heat and freezing-stress resistance varied considerably in various potato species (table 1; fig. 2). Three of the four noncultivated accessions (except *S. commersonii* 'Oka 5040') had relatively high frost resistance (-4 C or lower) and low heat resistance (1.5 to 3 h). The cultivated potato species *S. tuberosum*, on the other hand, had the least frost resistance (-2.5 C) and next to the highest heat resistance (table 1; fig. 2). However, *S. commersonii*

TABLE 1
FREEZING- AND HEAT-STRESS RESISTANCE OF FOUR POTATO SPECIES AS MEASURED BY
TTC AND CONDUCTIVITY TESTS

SPECIES	FREEZING-STRESS RESISTANCE AT 50% VALUE ($^{\circ}$ C) ^a		HEAT-STRESS RESISTANCE AT 50% VALUE (h) ^b	
	TTC test	Conductivity test	TTC test	Conductivity test
<i>Solanum tuberosum</i> 'Red Pontiac'.....	$-2.5 \pm .10$	$-2.5 \pm .05$	$7.4 \pm .50$	$7.0 \pm .45$
<i>S. multidissectum</i>	$-4.5 \pm .05$	$-4.0 \pm .05$	$1.5 \pm .10$	$1.8 \pm .10$
<i>S. commersonii</i> :				
'Oka 4583'.....	$-5.5 \pm .15$	$-5.3 \pm .10$	$3.0 \pm .25$	$3.2 \pm .30$
'Oka 5040'.....	$-4.7 \pm .20$	$-4.2 \pm .20$	$7.5 \pm .40$	$7.8 \pm .50$
<i>S. acaule</i>	$-6.0 \pm .20$	$-6.0 \pm .15$	$2.9 \pm .20$	$3.2 \pm .20$

NOTE.—Deviations were estimated from the plots similar to those in figs. 1–3. The standard deviation for the replicate means (bars in figs. 1–3) were considered as the maximum deviation around the 50% value.

^a The duration of freezing stress was 30 min at each freezing temperature.

^b The tissue was exposed to 45 C heat stress for various time periods.

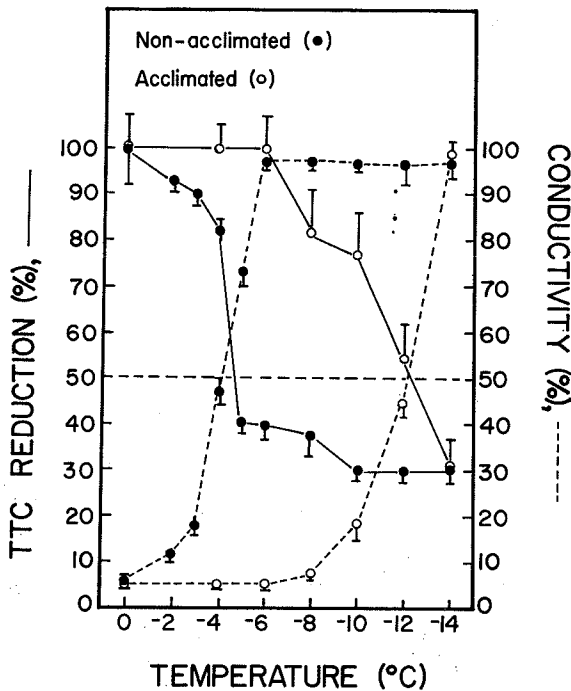


FIG. 1.—Estimated frost hardiness in acclimated and non-acclimated *Solanum commersonii* 'Oka 5040' by TTC reduction and conductivity tests. Nonacclimated plants were grown at a 20/15 C day/night. Plants were acclimated at 5/2 C, day/night regime for 15 days. The duration of freezing stress was 30 min at each freezing temperature.

'Oka 5040' had relatively high frost resistance and the highest heat resistance (table 1).

EFFECT OF COLD ACCLIMATION ON HEAT-STRESS RESISTANCE.—*Solanum commersonii* 'Oka 5040' had a frost resistance of about -4.2 C; the acclimated plant, -12.2 C (fig. 1). Among the other species, *S. tuberosum* had no increase in hardiness; *S. commersonii* 'Oka 4583' plants changed from -5.5 to -11 C; *S. multidissectum* had a 4 C increase from -4 C; and *S. acaule* had a 3 C increase from -6 C by cold acclimation.

After some preliminary trials with all of the species, we determined the effect of cold acclimation on heat-stress resistance in *S. tuberosum* 'Red Pontiac' and *S. commersonii* 'Oka 5040.' Results of the heat-resistance tests on these cold acclimated plants were compared with the nonacclimated controls (fig. 3). Heat-stress resistance of *S. tuberosum* either did not change or decreased slightly, depending on the viability tests used (fig. 3A). The conductivity test showed no change in heat-stress resistance of *S. tuberosum* after cold treatment. The TTC test, however, showed a decrease in the heat resistance of *S. tuberosum* from 24 to 44 min after cold treatment (fig. 3A). This species had no change in freezing-stress resistance after cold treatment.

Heat resistance of *S. commersonii* 'Oka 5040' almost doubled after cold treatment (fig. 3B). The

time at which 50% of injury symptoms appeared increased after cold treatment from 58 to 88 min in the conductivity test and from 42 to 86 min in the TTC test (fig. 3B). This potato species had a threefold increase in freezing-stress resistance (-4.2 to -12.2 C) after exposure to cold treatment (fig. 1). Increase in heat resistance after cold acclimation treatment was also observed in *S. acaule* and *S. multidissectum* (data not shown).

Discussion

VIABILITY TESTS FOR HEAT- AND FREEZING-STRESS RESISTANCE.—Both TTC and conductivity tests have been used successfully to evaluate relative freezing-stress resistance of potato and other plant species (LI and PALTA 1978; PALTA, LEVITT, and STADELMANN 1978; CHEN, GAVINLERTVATANA, and LI 1979). Results in this study reveal that these tests satisfactorily evaluated relative heat-stress resistance of potato species. Since TTC reduction occurs as it accepts electrons from the electron-transport chain (NACHLAS, MARGULIES, and SELIGMAN 1960), it is probably an indication of the status of enzymes involved in the oxidation-reduction reactions in the cell. Conductivity, on the other hand, is a measure of ion efflux, primarily K^+ , following alteration in the cell membrane properties (PALTA, LEVITT, and STADELMANN 1977b). A rapid increase in ion efflux after injury was due to the inactivation of the transport system (membrane intrinsic proteins), following which a passive efflux of ions results (PALTA et al. 1977b; PALTA and LI 1978, 1980). It appears, therefore, that enzymes that participate in oxidation-reduction reactions and active transport of ions are sensitive to heat injury.

RELATIVE SUSCEPTIBILITY OF DIFFERENT POTATO SPECIES TO HEAT AND FREEZING STRESS.—Field observations and some studies on the seasonal changes of heat resistance in *Dactylis glomerata* indicated that resistance to heat and freezing stress is mutually exclusive (FELDMAN and KAMENTZEVA 1963; LEVITT 1972). Our results show that such a generalization cannot be made. *Solanum commersonii* 'Oka 4583' and 'Oka 5040' had about the same level of frost resistance but had more than twofold differences in heat resistance; also, *S. tuberosum* had a lower level of frost resistance and about the same level of heat resistance as *S. commersonii* 'Oka 5040.'

The variations in heat and frost resistance among potato species are consistent with their ecological distributions. PALTA and LI (1979) explained differences in freezing-stress resistance among several potato species in terms of adaptation to various climatic and geographical variations by natural selection. Two species, *S. acaule* and *S. multidissectum*, are adapted to the high Andes and possess a high degree of frost resistance but a relatively low degree of heat resistance. *Solanum tuberosum*, thought to have originated on the islands off the

coast of Chile, has a relatively high degree of heat-stress resistance but little freezing-stress resistance. *Solanum commersonii* is distributed near the coastal region of Argentina where the temperatures are relatively high in the summer and relatively cool (freezing) in the winter; it possesses both high heat and frost resistance. *Solanum commersonii* 'Oka 5040' has both high heat and frost resistance, but *S. commersonii* 'Oka 4583' has a high frost and a low heat resistance. This probably means that factors other than climatic adaptation are also important in determining variations in heat and freezing-stress resistance between different accessions of a species.

EFFECT OF COLD ACCLIMATION ON HEAT-STRESS RESISTANCE.—There have been some reports of increased heat and frost resistance in plants during cold acclimation in the fall and winter by ALEXANDROV (1964), who explained this simultaneous increase in terms of an increase in the thermostability of proteins due to the direct action of low temperatures on the plants. LEVITT (1972) discussed these results and pointed out that two factors may be in-

involved during cold acclimation: (1) an increase in avoidance of protein aggregation and (2) an increase in the ability to repair the damage following stress. Our results (fig. 3) support ALEXANDROV's conclusion and show that a simultaneous increase in heat and frost resistance in some potato species can be achieved within 2 wk of cold acclimation. In the cultivated species no increase in resistance to either heat or freezing stress was found (fig. 3).

Based on the discussion by LEVITT (1972) and ALEXANDROV (1964), the difference between *S. tuberosum* and *S. commersonii* 'Oka 5040' suggests that, if the plant is capable of increasing thermostability of the proteins involved in freezing-stress resistance, it also seems to have the ability to increase thermostability of those involved in heat-stress resistance. These and our results also suggest that the ability to acclimate to cold is not directly related to the basic heat resistance level of the non-acclimated plant. Further studies with these potato species may provide more information on the nature of the relationship between heat and freezing stress.

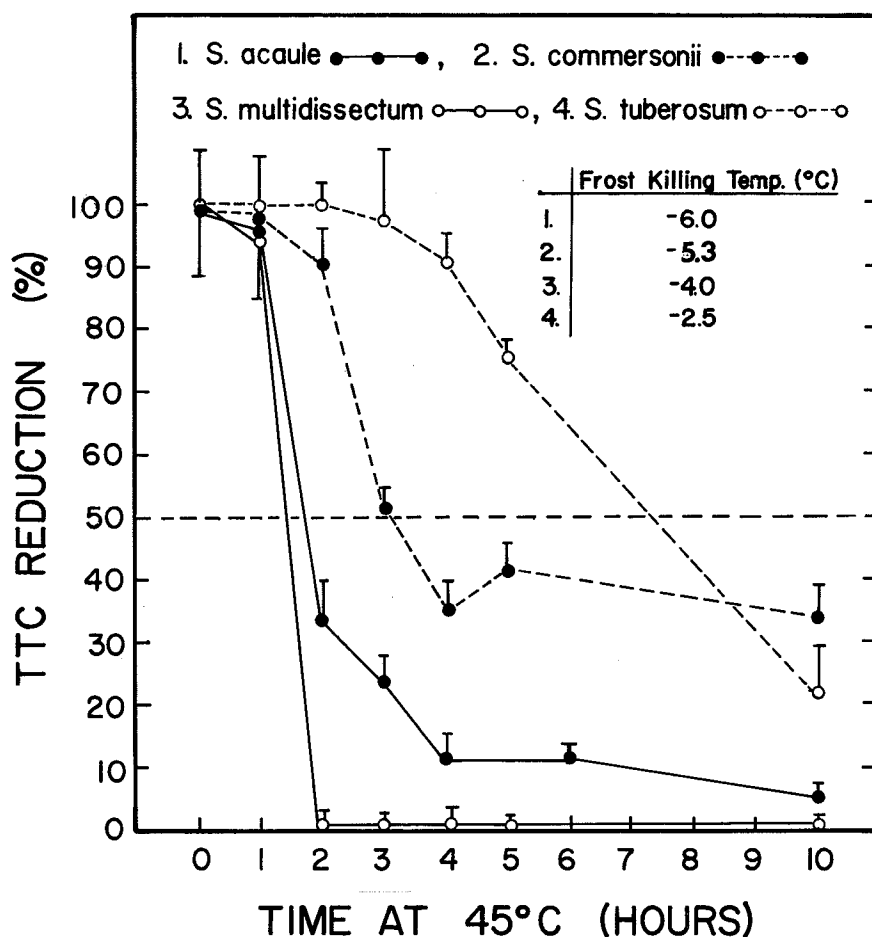


FIG. 2.—Differences in heat-stress resistance in tuber-bearing *Solanum* potato plants grown in a regime of 14-h photoperiod and 20/15 C day/night.

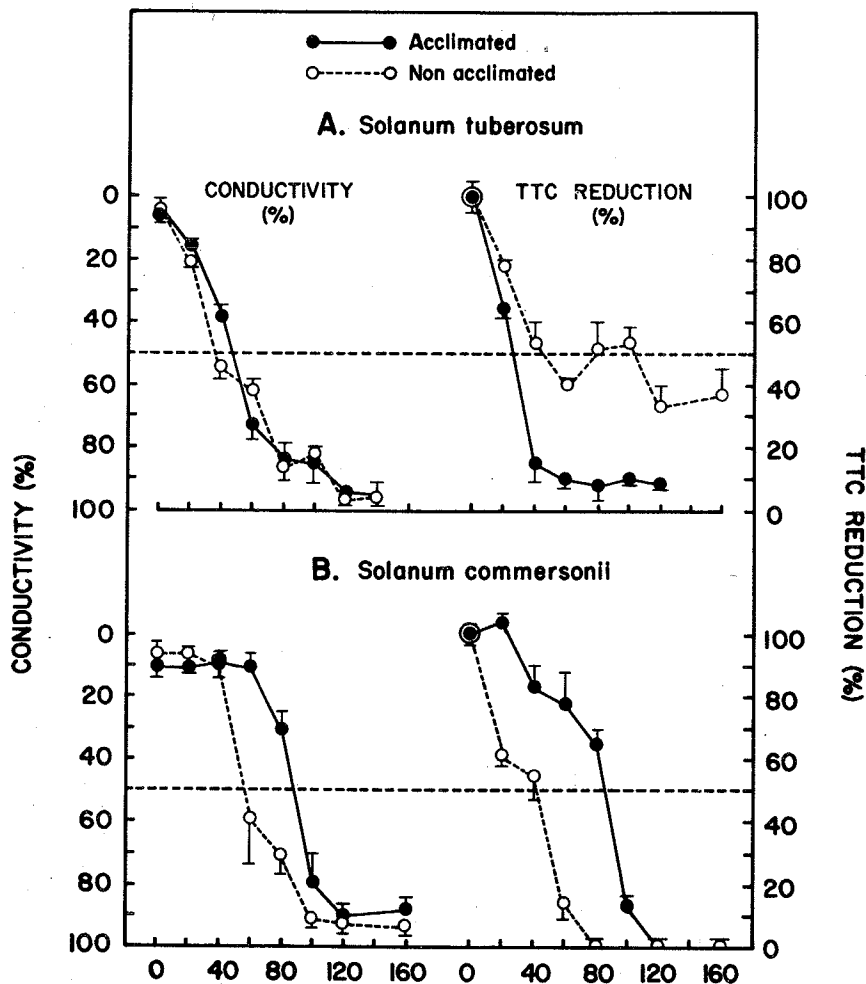


FIG. 3.—Effect of cold-acclimation treatment on heat-stress resistance in plants of *Solanum tuberosum* (A) and *S. commersonii* 'Oka 5040' (B). Growing conditions were the same as in fig. 1. Leaf tissues were exposed to 50 C heat stress for different time periods.

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