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Chilling injury in husk tomato leaves as defined by scanning calorimetry

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Abstract

Chilling injury is a common disorder to both tropical and some temperate species. Husk tomato plants (*Physalis ixocarpa* L.) were used to study chilling injury and define temperature ranges at which such injury occurs. Greenhouse-grown 'Cerro Gordo' husk tomato plants were used. Samples were taken from the first true leaf of 1-month-old plants. Preliminary tests using electric conductivity (EC) were carried out to narrow a temperature range. Results indicated that 1 h exposure between 6 and 3°C was enough for leaf tissue to increase ion leakage. Afterwards a differential scanning calorimetric assay was done in a range from 15 to 0°C and a scanning rate of 7°C h^{-1}. To include the effects of period and temperature exposure, a factorial experiment was carried out with exposures from 0 to 3 h at half an hour intervals, and isotherms were done at 0, 3, 6, 9, and 12°C. Activity recovered (%) was estimated by comparison to activity at 25°C. A highly significant interaction between exposure and temperature was found, confirming EC results. On average, exposures above 1 h and temperatures below 6°C caused the lowest recovery in activity as defined by the algorithm Actrec (%)=(97−16t+2.5T)/(1−0.01t+0.02T) with an R^2 of 0.95. Since a clear temperature breaking point was not observed, an analysis of scanning data was done. First by a fourth order polynomial regression to fit such data, followed by a second derivative to estimate inflection points associated with slope changes. Such inflections are regarded as changes in tissue metabolic activity, and our results clearly narrow such point ca. 5°C. Therefore, we can conclude that conditions leading to the lowest recovery in metabolic activity include exposures to temperatures at or below 5°C for 1 h or more.

Keywords: Chilling injury; Husk tomato; Calorimetry; Ion leakage

1. Introduction

Chilling injury is a physiological stress on plants, which leads to diminished quality and losses in product utilization, after exposures to low but non-freezing temperature [1]. In general, commercial vegetable crops cultured in tropical and temperate regions, might suffer considerable damage within 10 and 0°C. This type of event has been associated with changes in lipid composition, differences within species and membrane proteins. The more cold sensitive species have a higher proportion of saturated fats, which affects the membrane exchange capacity and functional fluidity [2,3,4].

On the other hand, some authors mention that some viability tests for chilling injury are more suitable for

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one plant species than another [5,6,7,8,9,10]. Where possible, using several viability tests simultaneously minimizes problems in determining chilling injury. In addition, Anderson et al. [11] made two relevant remarks; first, laboratory simulated cold episodes are different than those occurring in nature; laboratory episodes are primarily isothermal processes. Second, the time of exposure and sample mass should be included in the experimental design. Previous studies for calorimetrically determining plant tissue respiration, growth, and responses to thermal extremes as a function of time and temperature [12,13], are referred by sample dry mass and have settled basis for quantitative assessment of plant performance under distinct environmental conditions. The inactivation of tomato cells (*Licopersicum esculentum* L.; *L. peruvianum* L.) at high and low temperature was determined by Rank et al. [14] using this technique, they proposed a cycling method for evaluating in vivo responses to temperature stress.

The primary objective of the present study was to determine the temperature and time of exposure leading to chilling injury in husk tomato plants (*Physalis ixocarpa* L.) complementing conventional EC tests with calorimetric methods.

2. Materials and methods

Leaf tissue of greenhouse-grown husk tomato plants (*P. ixocarpa* L.) var. 'Cerro gordo' was used. Leaves were 2 to 3 cm long and round in shape.

Cold stress tests were carried out with a Neslab RTE-140 circulating bath, on discs from the first true leaf. Discs, 6.35 mm in diameter, were punched avoiding the blade central nerve. Ten leaf discs were wrapped in cheese cloth and aluminum foil for each treatment. All of the treatments were packed in a ziplock plastic bag and submerged in a 40% ethylenglycol bath at 12, 9, 6, 3, and 0°C, with 1 h exposure. Damage was evaluated as the percent of ion leakage for each treatment.

Right after the cold episode, leaf discs were incubated at 4°C during 12 h. Afterwards, EC measurements were carried out. Samples were suspended in 25 ml of deionized water, shaken overnight (≈10 h). Then, the solution conductivity was measured with an Orion benchtop conductivity meter model 160 (accuracy of +0.5%, Orion Research, Inc., Beverly MA) data were expressed as μs/cm (mmhos/cm). Afterwards, the samples were heat treated (60°C for an hour), cooled to room temperature and volume corrected due to evaporation. They were agitated overnight and final conductivity was determined. Data were registered as percent of conductivity.

Isothermal and differential scanning were carried out according to the methods developed by Rank et al. [14]. A CSC 4100 model DSC was used (Calorimetry Science Co., Pleasant Grove, UT). This instrument has four hermetic hastelloy ampoules of 1 cm^3, sensitivity of +1 μW, and its scanning capacity ranges from -30 to 110°C. The fourth ampoule was kept empty during the experiment as a reference.

A constant N_2 flux of 175 g cm^{-2} was circulated to prevent moisture from condensing in the instrument. A circulating bath was used (PolyScience, USA) to keep the temperature constant at 15°C.

Leaf tissue was rinsed with sterilized water. Excessive moisture was eliminated and fresh weight was recorded. Afterwards, whole leaves were introduced to their corresponding ampoules.

For isothermal calorimetry, five isotherms were used; 12, 9, 6, 3 and 0°C each was 30 min in duration, followed by a 25°C isotherm to determine percent of recovery. Data were baseline adjusted and reported as a function of dry weight.

Scanning conditions ranged from 15 to 0°C at 7°C/h, data were recorded every 30 s. Data obtained were baseline corrected and expressed as a function of their dry weight. Heat rate data were transformed to natural logarithm and temperature was expressed as T/K, for an Arrhenius plot. Further statistical analysis included a second derivative of a fourth order polynomial regression to identify inflection points along the curves.

Arcsine transformed data from EC and isothermal measurements were statistically analyzed by an ANOVA, and mean separation was carried out by Tukey's test ($p<0.05$). Statistical analysis was performed using SAS 6.08 version [15].

3. Results and discussion

Ion leakage from husk tomato leaf tissue at each of the temperature treatments is shown in Fig. 1. A third

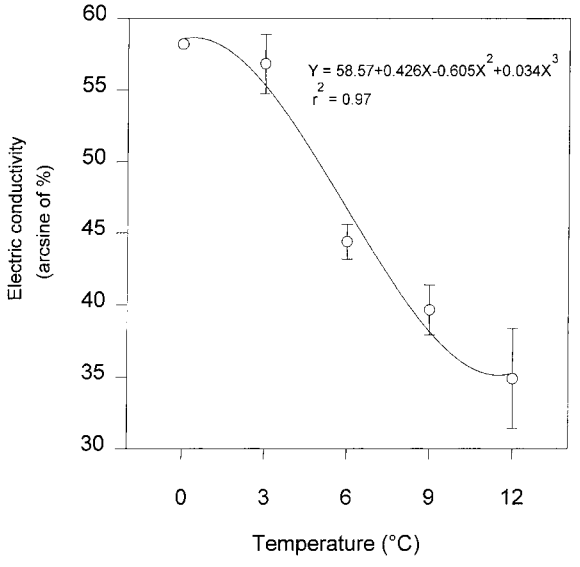

Fig. 1. Electric conductivity response of husk tomato leaf discs subjected to chilling temperatures.

order polynomial with a high coefficient of determination (R^2=0.97) shows that significant increases in ion leakage occurred between 9 and 6°C and below 6°C; this condition indicates chilling injury. Since the temperatures within these ranges were not measured, we could not determine the precise point at which the injury occurred.

The activity recovered after exposures at chilling temperatures for different periods is a complex function of the highly significant interaction between these two factors. The isothermal results presented here suggest that chilling injury was triggered by temperatures in a range from 6 to 3°C and exposures of at least 1 h, as shown in Fig. 2. The response surface described by the experimental data points is fitted by the equation

$$\text{Actrec}(\%) = \left(\frac{97 - 16t + 2.5T}{1 - 0.01t + 0.0T}\right)$$

where t is time (h) and T is temperature (°C), R^2=0.95.

A graphical analysis of such figure concludes that at any given temperature, exposures beyond 1 h resulted in highly significant decreases in activity. These data agree with EC results, narrowing the temperature threshold closer to 6°C. Therefore, in this test, exposures to 6°C for over 1 h resulted in the most drastic reduction in activity recovered. Of course conditions exceeding the above caused further losses in tissue activity.

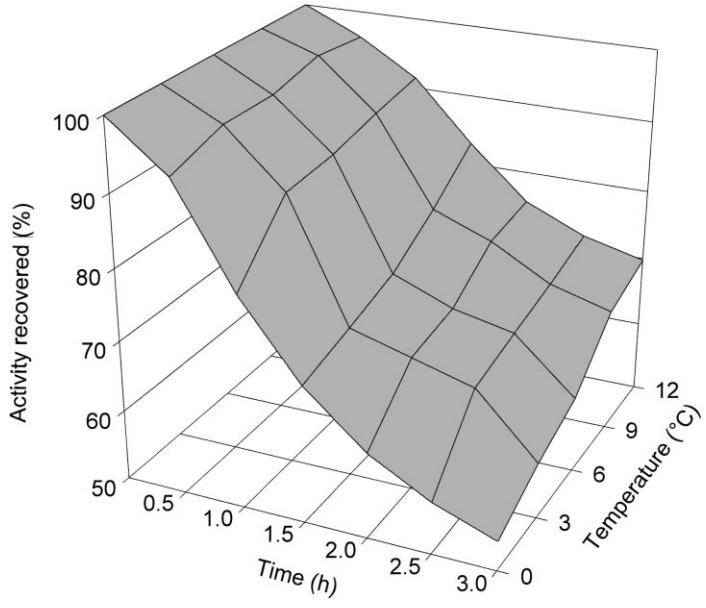

Fig. 2. Activity recovery in husk tomato leaf tissue as a response to different temperature-time interactions, as defined by the algorithm % Actrec=(97−16t+2.5T)/1−0.01t 0.02T) with an R^2 of 0.95. Standard error for the difference between two means, 1.7 (p<0.001).

Table 1
Fourth order polynomials fitting scanning data of metabolic heat rate as a function of temperature, and curve inflection points as determined by a second derivative analysis

Algorithm	R^2	Inflection points (°C)	
$y=-2845122.7+3180826.598\ x-1333526.139\ x^2+248469.3\ x^3-17360.7x^4$	0.99	7.5	5.2
$y=-4347778.88+4864960.95\ x-2041309.51\ x^2+380664.9\ x^3-26619.2x^4$	0.98	8.1	5.1
$y=-6637956.4+7418415.6\ x-3108892.8\ x^2+579035.1\ x^3-40441.1x^4$	0.96	7.7	4.7

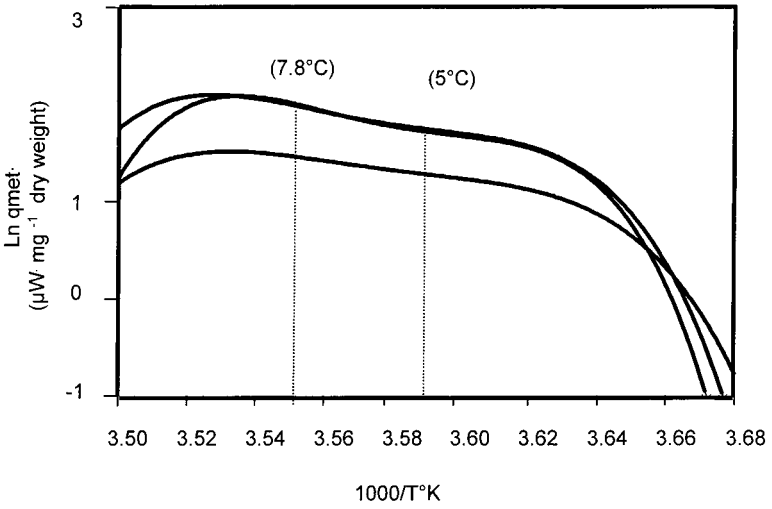

Fig. 3. Arrhenius plots for metabolic heat rate of leaf tissue from husk tomato. Dotted lines show average inflection points as determined by a second derivative analysis.

Fourth order polynomial regression parameters for three DSC data sets are shown in Table 1. The average inflection points for these three lines, as defined by a second derivative analysis, were 7.8 and 5°C. The segments within such range are linear (see Fig. 3), which means that the apparent activation energy maintains a constant value. As an example, above 7°C the tissue may perform its normal vital functions. However, the inflection point at 5°C represents the end of the linear behavior and beyond that, tissues start to experience inactivation [13]. Such condition is valid only for the scandown rate used (7°C h^{-1}), since this point may change its value depending on the rate magnitude, which shows a time of exposure dependency [13,14]. Although, these scans do not represent a thermal dependence in real time; nevertheless, they still represent a useful reference for screening chilling sensitive species or conditions leading to chilling injury development.

Further studies involving membrane integrity and functionality may be considered to complement calorimetric information in order to achieve conclusive results.

4. Conclusions

Conditions for chilling injury in husk tomato plants (*P. ixocarpa* L.) are exposures to temperatures below 5°C for at least 1 h. Our results agree with those reported elsewhere [14], as far as establishing accurately and rapid in vivo measurements of metabolic activity shifts related to injury, condition that EC cannot render.

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