Short Communication

Short Term Increases in the Cold Tolerance of Red Osier Dogwood Stems Induced by Application of Cysteine

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ABSTRACT

Bark tissues of Cornus stolonifera stems, treated with cysteine at 24 hours after treatment, survived exposure to −11 C (the tissue temperature) with little or no injury. An initiation of increase in the cold tolerance was usually observed when plants were treated with cysteine at 12 hours after treatment. Neither plants at 36 or 48 hours after treatment nor plants 12 hours before treatment had shown increases in the cold tolerance. They were killed below −5 C, which was the survival temperature of untreated control plants. Two weeks or more of short day induction before cysteine application were required for a significant effect of short term 5 C increase in the cold tolerance.

An increase of a few degrees in cold tolerance at critical times could significantly attenuate frost injury and reduce losses in many crops. Efforts to identify chemical protectants capable of rapidly increasing tolerance for short periods of time without detrimental side effects have met with limited success. At one time or another, cold-protective properties have been attributed to a number of growth-regulating substances (3, 5, 6); sugars and polyhydric alcohols (3, 7); proline (3); and ascorbic acid (1). However, no chemicals are routinely used for crop cold protection. For the past several years, we have observed increased cold tolerance in our tests from field, greenhouse, and growth chambers in which red osier dogwood plants, Cornus stolonifera Michx., have been treated with L-cysteine (free base, obtained from Sigma Chemical Company) prior to freezing tests. No substance other than cysteine has been systematically tested for its ability to increase in cold tolerance for red osier dogwood plants.

Rooted cuttings of a single clone of dogwood were grown for 2 to 3 months in a greenhouse under a regime of long day (LD 16 hr) and warm temperatures (22–20°C day/18–16°C night). Uniform plants in part were then grown in a controlled environmental chamber for 2 weeks under a regime of short day (SD-10 hr) and warm temperatures (20°C day/15°C night). The foliage and stems of plants, preconditioned under SD regime for 2 weeks, and plants of the same age, which had remained in LD regime greenhouse, were then sprayed with 0.1 M cysteine. Excised stem sections from control plants and sprayed plants from both photoperiod pretreatment regimes (LD-SD) were subjected to controlled freezing tests at 12, 24, 36, and 48 hr after cysteine application.

In each freezing test, uniform stem sections (6–7 mm diameter/5–6 cm long) with thermocouples embedded in the pith were slowly cooled (3°C/hr) to various freezing temperatures of −5, −7, −9, −11, or −13°C, removed from the freezer and thawed slowly in a 0°C refrigerator, and incubated for 5 to 6 days in a humid chamber at room temperature. Viability of bark tissues after freezing was evaluated visually by examining intact and dissected stems microscopically and rating them for oxidative browning associated with freezing injury (4). Results of this viability test correlate well with intact plant regrowth in the greenhouse after freezing, a more direct means of evaluating survival. Application of cysteine to the plants grown under LD regime did not increase their cold tolerance.

Figure 1 illustrates the results of a typical test when stems from cysteine treated and untreated control plants from the SD regime were subjected at 24 and 48 hr after treatment to various freezing temperatures of −5, −7, −9, and −11°C. Bark tissues of stems from plants treated with cysteine at 24 hr after treatment survived exposure to −11°C (tissue temperature) with little or no injury. An initiation of increase in cold tolerance was usually observed when plants were treated with cysteine at 12 hr after treatment. Neither plants at 36 or 48 hr after treatment nor plants 12 hr before treatment had shown increases in cold tolerance. They were killed below −5°C, which was the survival temperature of untreated control plants.

Short day precondition seemed necessary for dogwood stems to respond to cysteine treatment. Figure 2 illustrates the relationship between the duration of short day induction and the effectiveness of cysteine in increasing cold tolerance. Results indicated that 2 weeks or more of SD induction before cysteine application were required for a significant effect of a 5°C increase in cold tolerance. It is known that SD per se induces cold acclimation in red osier dogwoods (8), but this response required more than 4 weeks of SD induction. Cysteine may function to enhance or accelerate this short day response.

The observations seemed in favor of supporting Levitt's (2) sulfhydryl hypothesis of freezing injury. According to this hypothesis, injury is caused by the formation of intermolecular disulfide bonding during freezing. Cysteine, a sulfhydryl containing amino acid, may function to prevent the SS formation, and

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2 Abbreviations: LD: long day; SD: short day.
Fig. 1. Observations of short term increases in the cold tolerance of excised stems of red osier dogwoods which were preconditioned under SD regime for 2 weeks. 24 hr and 48 hr: excised stems were subjected to controlled freezing tests at 24 and 48 hr after cysteine treatment, respectively. At 24 hr after application, stems could survive up to -11 C, while samples of 48 hr and control were killed below -5 C. a: alive; d: dead.
COLD TOLERANCE AND CYSTEINE

The capacity of cysteine to increase hardiness of stem tissues of red osier dogwood warrants further evaluation, particularly on crop species which acclimate slowly in response to shortening day length in the fall and on other plant tissues and organs.

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LITERATURE CITED