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Short Communication

Relationship between Mefluidide Treatment and Abscisic Acid Metabolism in Chilled Corn Leaves[1]

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ABSTRACT

Mefluidide, *N*-(2,4-dimethyl-5[([trifluoromethyl]sulfonyl) amino] phenyl)acetamide, a synthetic plant growth regulator, was capable of triggering an increase in endogenous free abscisic acid content when corn (*Zea mays* L.) plants were grown in a nonstress, day/night, temperature regime (26°C) with sufficient moisture supply. The relevance of such an abscisic acid increase prior to chilling exposure and the water relations during chilling are discussed in reference to the mefluidide protection of the chilled corn plants.

In 1984, Tseng and Li (13) first reported that mefluidide, a synthetic plant growth regulator (1), is capable of protecting corn plants from chilling injury. Subsequently, quantitative measurements of mefluidide protection of chilled corn leaves, and post-application growth and development were reported (14). During chilling, plants lose water, resulting in tissue weight loss (16), leaf necrosis (8) and over all growth reduction (16). It has been suggested that hormonal regulation may be involved in chilling resistance (6, 9, 15). ABA may aid plants in preserving better water balance (4, 7) and in tolerating more chilling stress (8, 10). The following reports on water relations and the metabolism of the ABA in corn leaves after mefluidide treatment before, during, and after chilling.

MATERIALS AND METHODS

Seeds of 'A632 × C103YU', a single cross corn hybrid (*Zea mays* L.), were sown into a soil/sand/peat (3:2:2, v/v) medium in 15-cm diameter pots. Seedlings were thinned to two per pot. Pots were kept in a growth chamber with a regime of 12-h photoperiod, 400–450 E m^{-2} s^{-1} PAR, and 26°C D/N[3] temperature. When plants had 3 to 4 true leaves, they were sprayed with analytic grade mefluidide at concentrations of 10 and 20 ppm (mg/L). The mefluidide was first dissolved in 2 to 3 ml of acetone, and then quickly diluted with distilled H_2O to an appropriate volume and a few drops of Tween 20 surfactant added. Foliar spray was stopped when leaf tips showed droplets.

Twenty-four h after treatment, plants were transferred from the chamber to a chilling room at 4°C D/N temperature and 12 h photoperiod. Plants were chilled for 6 d, and then were moved from the chilling room back to the chamber with 26°C, D/N, temperature and 12 h photoperiod. Plants were fully watered during chilling. Plants sprayed with water containing a few drops of Tween 20 and acetone served as the control.

Samples were collected at the 1st, 3rd, 5th, 7th and 9th d after mefluidide treatment. The first day samples were collected from plants that had been grown for 24 h at 26°C after treatment. The 3rd, 5th, and 7th d samples were collected from plants that had been chilled for 2, 4 and 6 d, respectively. The 9th d samples were collected from plants that had been grown at 26°C, D/N, for 2 d after removal from chilling.

ABA Measurements. ABA was extracted, purified and quantified according to the method of Schussler *et al.* (11). About 25 g (fresh weight) of leaf tissues (2nd true leaf) collected from 6 plants for each treatment at each sampling date were sliced and thoroughly mixed. Six replicates, 1 g each, were weighed out from the sliced tissues, and about 5,600 dpm of [^{3}H]ABA were added to each as an internal standard. Samples were homogenized in 10 ml of cold extracting medium (80% methanol plus butylated hydroxyltoluene, 10 mg/L). ABA analysis was carried out according to procedures described by Schussler *et al.* (3).

Water Relations. Leaf water potentials (WP), using the first true leaf, were measured with a pressure chamber. Seven measurements from each of 6 plants for each treatment were made at each sampling date. Leaf osmotic potentials were measured with a vapor pressure osmometer. Discs were punched from the 3rd true leaf. Leaf sap was pressed from the disc and collected with a filter-paper disc which was then placed in an osmometer. OP was calculated according to OP = iCRT, where i = isosmotic coefficient, C = leaf sap concentration, R = gas coefficient, and T = absolute temperature. Seven OP measurements from each of 6 plants were made for each treatment at each sampling date. RWC was calculated according to RWC = (fresh weight − dry weight/(turgid weight − dry weight). Turgid weight was obtained by placing the cut end of the first true leaf in a beaker containing water. The beaker was enclosed in a humid-atmosphere chamber for 12 h at either 26°C or 4°C ambient temperatures, corresponding to the plant-grown temperatures. Submerged ends of leaves were blotted rapidly before weighing. Leaves were dried in an oven at 120°C for about 48 h and dry weights determined.

RESULTS

ABA Content. Endogenous free ABA in controls and mefluidide-treated plants before, during and after chilling is shown in Figure 1. During the first 24 h period after mefluidide treatment at 26°C, control plants had a constant ABA content of 28, ng/g

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[3] Abbreviations: D/N, day/night; OP, leaf osmotic potential; WP, leaf water potential; RWC, relative water content.

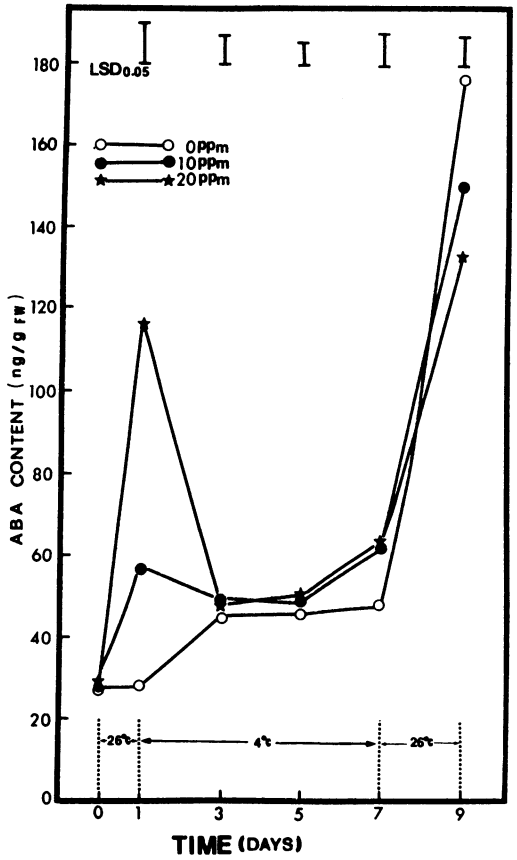

FIG. 1. Changes in ABA content in mefluidide-treated corn leaves before, during and after chilling. Data are means of six replicates.

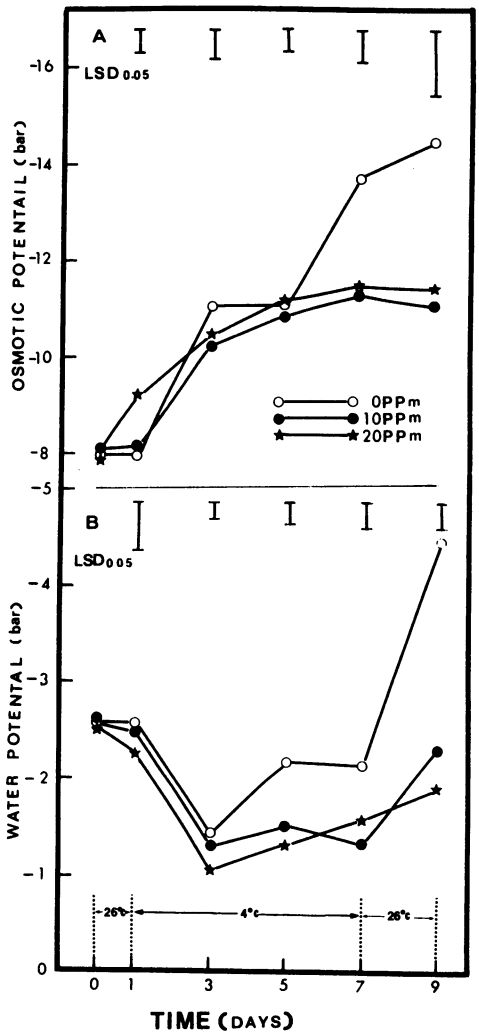

FIG. 2. Leaf osmotic potentials (A) and leaf water potentials (B) of mefluidide-treated plants before, during and after chilling. Data are means of seven replicates.

fresh weight, whereas, plants treated with 10 and 20 ppm mefluidide increased to 57 and 117, respectively.

Two d after chilling, ABA in the control increased from 28 to 45 ng/g. The 10 ppm treated plants held a level at 50 similar to that observed at the warm regime. ABA content decreased in 20 ppm treated plants when plants were chilled for 2 d. During 4- and 6-d chilling, 10 and 20 ppm treated plants, as well as controls, maintained a similar and constant level of ABA.

When plants were removed from chilling and placed at the 26°C regime, ABA content significantly increased in treated as well as in control plants. The control, which had the greatest chilling damage, showed the highest increase in ABA content, whereas 10 and 20-ppm treated plants had levels of 130 and 150 ng/g, respectively.

Water Relations. The WP in both control and treated plants increased about 1.5 bar after 2 d chilling (Fig. 2B). Thereafter, a more or less constant level of −1.0 to −2.0 bars was observed between the control and treated plants. After removal from chilling, WP of the control plants declined as compared with treated plants in which the decreases were insignificant. Changes in OPs were similar to the WPs between controls and treated plants (Fig. 2A). Plants treated with mefluidide showed no significant changes in RWC during and after chilling, whereas the control continuously decreased in RWC (Fig. 3).

DISCUSSION

Exposure of corn seedlings to chilling (4°C) caused drying of leaf margins and leaf necrosis. In general, the second and third true leaves were the most sensitive to chilling injury. On a per plant basis, about 60% damage (drying of leaf) to total leaf area resulted when controls were subjected to 4°C (D/N) for 6 days (14). However, leaves treated with 10 and 20 ppm mefluidide had only 14 and 12% damage, respectively, after the same duration of chilling (14). Plant growth was stunted from a foliar application of 20 ppm, though plants were protected from chilling injury. Growth reduction with high mefluidide concentration was accompanied by necrosis of leaf tips and plant distortion. Leaves failed to unroll, giving a 'buggy whip' appearance (14). For the corn hybrid A632 × C103YU, the optimum concentrations for chilling protection with no adverse effect on growth and development is about 5 to 10 ppm (14).

Although there is evidence that endogenous ABA increase is interrelated with the increase in plant cold hardiness (3), the changes are not necessarily parallel. In tuber-bearing *Solanum* species, ABA increase during low temperature exposure occurs prior to the increase in hardiness, and the increase is species-specific (3). Also, exogenously applied ABA has been demonstrated to ameliorate chilling injury (8, 10).

In this study, corn leaves doubled the amount of ABA content 2 d after chilling (Fig. 1). However, the plants showed more than 60% leaf damage after a 6 d chilling exposure. It appears that the increase in ABA during chilling, even at the early chilling stage, was not correlated to corn chilling resistance. Rikin *et al.* (10) has shown that ABA applied at the inception of the chilling was ineffective in preventing chilling injury, and application at

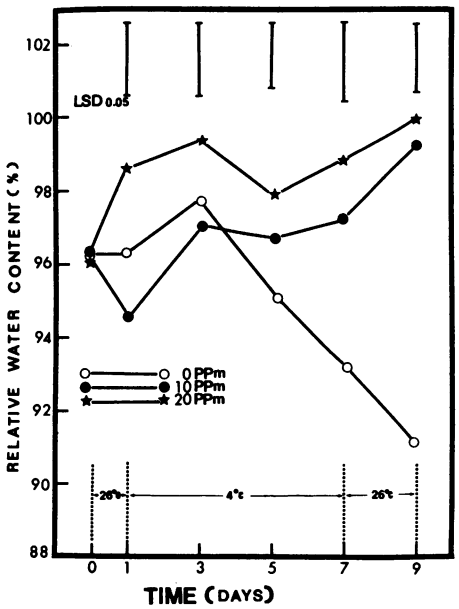

FIG. 3. Leaf relative water contents of mefluidide treated plants before, during and after chilling. Data are means of seven replicates.

least 3 h prior to chilling treatment was required in order to have a positive effect. We did not observe significant chilling protection of the corn leaves unless mefluidide treatment was given in a warm regime for 12 h prior to chilling exposure.

Mefluidide seems capable of triggering ABA increase in corn plants with absence of chilling. The higher the dose applied, the greater the amount of ABA increase (Fig. 1). Chilling temperature also caused an increase in ABA content (the control). High dose treated plants also showed a decrease in ABA content during chilling. Changes in ABA content during chilling are likely influenced by the low temperature *per se* rather than by the low temperature-induced water stress (Figs. 2, A and B, and 3). It is known that under water stress ABA content increases (5). Results suggest that the increase in endogenous ABA, as triggered by mefluidide, before chilling would be a necessary step in order to activate the protection mechanism.

When plants were transferred from a chilling regime to a warm regime, both the control and treated plants had markedly increased in ABA content (Fig. 1). ABA increase in the control may be due to the change in temperature-induced water loss (Fig. 3) (2, 17). However, in mefluidide-treated plants, changes in water relations were insignificant (Figs. 2, A and B, and 3).

Results suggest that mefluidide is capable of triggering ABA synthesis in corn plants when they are growing at optimum temperature (26°C) with adequate water supply. The increase in ABA may then activate a protective system that enables the plant to minimize the injury during low temperature exposure. Interestingly, it has been demonstrated that ABA is able to induce synthesis of certain protein species. During seed maturation, for example, the accumulation of major storage proteins is controlled by the ABA metabolism (12). Evidence from this laboratory also indicates that an elevation of ABA may induce protein synthesis responsible for the increase in potato cold hardiness (3).

The timing of elevated ABA content (prior to chilling) appears to be more important for plant protection than the high level of ABA that occurs during chilling. ABA increased in the control during early stage of chilling (Fig. 1), but the plants did not show any protection from chilling injury (14). For chilling protection in corn, mefluidide spraying should be given at least 12 h prior to chilling exposure.

The obvious question is, if mefluidide acts by increasing ABA, why not just treat plants with ABA? There is evidence that ABA is capable of preventing plant injury from low temperatures (3, 8, 9). However, spraying the corn with ABA does not work. Another merit of applying mefluidide *versus* ABA is that mefluidide is economically feasible for large scale agricultural application.

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