J Plant Growth Regul (1998) 17:147–151

Journal of
Plant
Growth
Regulation

Involvement of Abscisic Acid in Mesocotyl Growth in Etiolated Seedlings of a Foxtail Millet Dwarf Mutant

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Received October 27, 1997; accepted May 11, 1998

Abstract. Etiolated seedlings of foxtail millet (*Setaria italica* Beauv.) dwarf mutant CH84113 were treated with various concentrations of abscisic acid (ABA), mefluidide, mannitol, or polyethylene glycol (PEG) 6000. It was found that these chemicals, at suitable concentrations, could increase mesocotyl length significantly, whereas these chemicals at higher concentrations had an inhibitory effect. Endogenous levels of ABA in mesocotyl were measured by enzyme-linked immunosorbent assay. It was found that endogenous ABA increased progressively in a chemical (ABA, mefluidide, mannitol, or PEG 6000) concentration-dependent manner, indicating that the effects of these chemicals on mesocotyl growth may be mediated by increased endogenous ABA levels. On the other hand, S-3307, an inhibitor of the oxidative reactions in gibberellin (GA) biosynthesis, inhibited the elongation of mesocotyl significantly. When ABA and GA_3 were applied simultaneously, the effect on mesocotyl growth was additive. These results imply that ABA and GA may control different processes in the regulation of mesocotyl growth.

Key Words. Foxtail millet—Mesocotyl—Abscisic acid—Gibberellin—Dwarf mutant

ABA is a particularly interesting hormone with regard to regulation of its levels, because they rise and fall dramatically in several kinds of tissues in response to environmental and developmental changes (Walton and Li 1995). Although exogenous applications can inhibit growth in the plant, ABA appears to act as much as a promoter as an inhibitor, and a more open attitude toward its overall role in plant development is warranted (Davies 1995, Lee and Zhou 1996). It has been found that ABA counteracts the effect of gibberellin (GA) on α-amylase synthesis in germinating cereal grains (Jacobsen et al. 1995). ABA also plays roles in physiological events occurring at the maturation events of embryo development (Rock and Quatrano 1995) and in the regulation of gene expression (Chandler and Robertson 1994). Here we report the promotive effect of ABA on the growth of mesocotyl in etiolated seedlings of a foxtail millet (*Setaria italica* Beauv.) dwarf mutant. We also utilize enzyme-linked immunosorbent assay (ELISA) to attempt a quantitative analysis of endogenous ABA in the mesocotyl. A proposed mechanism of mesocotyl growth is discussed.

Abbreviations: ABA, abscisic acid; GA, gibberellin; PEG, polyethylene glycol; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; IAA, indole-3-acetic acid.

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Materials and Methods

Culture of Etiolated Seedlings

Sterilized seeds of foxtail millet (*S. italica* Beauv.) dwarf mutant CH84113 were cultured in darkness at 25°C until radicle emergence. The cultured seeds were then planted in a 12-cm × 2.5-cm glass container containing 750 mg of absorbent cotton and 5 mL of water or test solution. After 4 days in darkness at 28°C, the length of the mesocotyl was measured. Data were the averages of three replicates ± S.E.

Application of Chemicals

Both ABA and GA_3 were applied at final concentrations as 0.005, 0.02, 0.08, 0.31, 1.25, 5, and 20 μM. Mefluidide was applied at 0.05, 0.20, 0.78, 3.12, 12.5, 50, and 200 μM. Mannitol was applied at 0.025, 0.10, 0.39, 1.56, 6.25, 25, and 100 mM. Polyethylene glycol (PEG) 6000 was applied at 0.005, 0.02, 0.08, 0.31, 1.25, 5, and 20 mM. S-3307 was applied at 0.025, 0.10, 0.39, 1.56, 6.25, 25, and 100 μM. Five mL of the

test solution was added to the container before the cultured seeds were planted.

In the ABA plus GA_3 treatment, ABA and GA_3 solutions at the same concentrations (0.005, 0.02, 0.08, 0.31, 1.25, 5, and 20 μM) were added simultaneously to the container before the cultured seeds were planted.

Sampling for ABA Analysis

Three days after planting cultured seeds, the mesocotyl was excised from the etiolated seedlings and washed thoroughly with distilled water. Fresh samples (0.10–0.20 g) were weighed, frozen in liquid nitrogen, then kept at −20°C until extraction of ABA analysis.

Extraction and Measurement of ABA by ELISA

Extraction and purification of ABA before immunoassay have been described previously (Chen et al. 1996a, Weiler 1986, Zhou et al. 1996). The main steps are as follows. Homogenized samples were extracted in 2 mL of cold 80% (v/v) aqueous methanol overnight at 4°C with butylated hydroxytoluene (10 mg/liter) to prevent oxidation. The supernatant was collected after centrifugation at 10,000 × *g* (4°C) for 20 min. Then the crude extract was passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA). The efflux was collected and 300 μL of it removed and dried in N_2. The residue was dissolved in 200 μL of PBS (0.01 M, pH 9.2), adjusted to pH 8.5, before partitioning three times with equal volumes of ethyl acetate. The remaining aqueous phase was adjusted to pH 2.5 and reextracted three times with equal volumes of ethyl acetate. The extracts (ethyl acetate phase) were pooled and dried under N_2; the residue was redissolved in 200 μL of 100% methanol for methylation with ethereal diazomethane and taken up with 300 μL of PBS (0.01 M, pH 7.4) for determining the ABA content.

The procedure of direct competitive ELISA measurement based on a monoclonal antibody of high specificity for ABA methyl ester has been described by Zhou et al. (1996). The main steps are as follows. Microtitration plates (Nunc) were precoated overnight at 4°C with rabbit antimouse immunoglobulin. Then the wells were coated with suitable amounts of anti-ABA methyl ester monoclonal antibody in PBS (0.01 M, pH 7.4) at 37°C for 70 min. Authentic ABA methyl ester or sample was added 30 min before the addition of horseradish peroxidase-labeled ABA. After a 1-h incubation at 37°C, the wells were washed five times with PBS (containing 0.05% Tween 20). Then the buffered enzyme substrates (H_2O_2 and *ortho*-phenylenediamino) were added, and the enzyme reaction was carried out in darkness at 37°C for 15 min. The reaction was terminated with 3 M H_2SO_4, and the absorbance was recorded at 490 nm.

The need to validate immunoassays for plant hormones has been well documented (Pengelly 1985). In this study, percentage recovery was calculated by adding a known amount of authentic ABA to the split extracts. Percentage recovery was above 90%, and sample extract dilution curves paralleled the standard curve, indicating the absence of nonspecific inhibitors in the extracts. ABA was determined three times on the same extract, and all samples were assayed in triplicate. The S.E. was calculated.

Results

Effects of Chemicals on Mesocotyl Growth

As shown in Fig. 1, ABA at a low concentration could promote the growth of mesocotyl in etiolated seedlings of CH84113. A significant increase in mesocotyl length

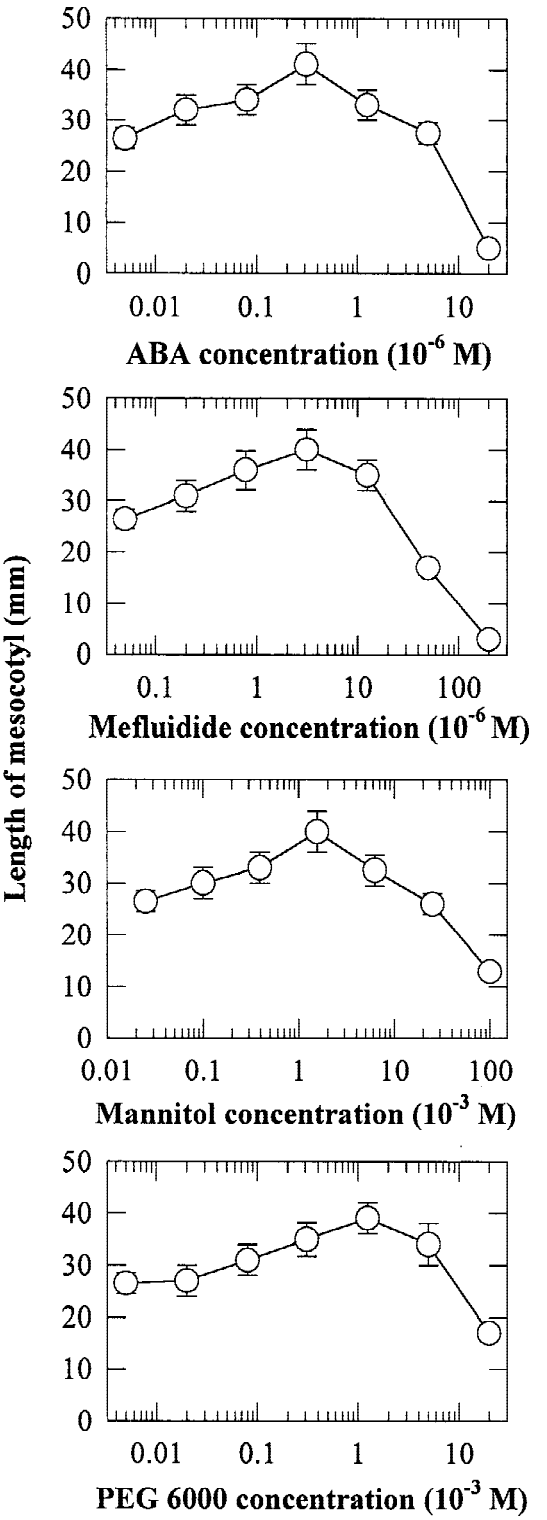

Fig. 1. Dose-response curves for ABA, mefluidide, mannitol, and PEG 6000, in terms of the length of mesocotyl in etiolated seedlings of CH84113. Data were collected 4 days after treatment. Shown are the averages ± S.E. of three replicates. The length of mesocotyl in untreated seedlings was 26.5 ± 2.0 mm. *Vertical bars* denote S.E. when larger than symbols.

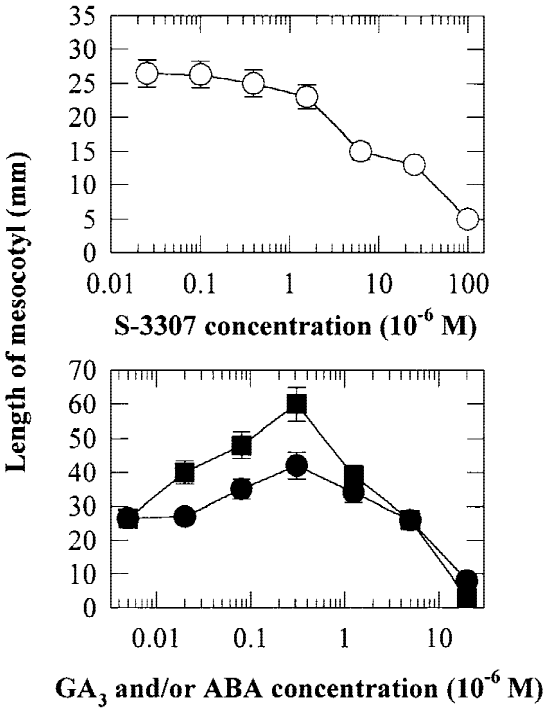

Fig. 2. Dose-response curves for S-3307 (○), GA_3 (●), and GA_3 plus ABA (■), in terms of the length of mesocotyl in etiolated seedlings of CH84113. Data were collected 4 days after treatment. Shown are the averages ± S.E. of three replicates. The length of the mesocotyl in untreated seedlings was 26.5 ± 2.0 mm. *Vertical bars* denote S.E. when larger than symbols.

was observed when ABA was applied at 0.31 μM. The length of mesocotyl increased by approximately half. However, when the ABA concentration was larger than 5 μM, mesocotyl growth was inhibited.

The dose-response curve for mefluidide was similar to that of ABA, with the most significant increase observed when mefluidide was applied at 3.12 μM (Fig. 1). At 50 μM, mefluidide was inhibitory, and at 200 μM, the mesocotyl could hardly grow.

Mannitol could also elicit a significant increase in the growth of mesocotyl when it was applied at concentrations lower than 25 mM (Fig. 1).

When PEG 6000 was applied at concentrations lower than 5 mM, the mesocotyl length increased in a PEG 6000 concentration-dependent manner (Fig. 1). PEG 6000 at 20 mM inhibited the growth of the mesocotyl significantly.

The promotive effect on mesocotyl growth was not observed when S-3307, an inhibitor of the oxidative reactions leading from *ent*-kaurene to *ent*-kaurenoic acid in GA biosynthesis (Hedden 1990, Izumi et al. 1984), was applied (Fig. 2). Mesocotyl elongation decreased in an S-3307 concentration-dependent manner. When S-3307 was applied at 25 μM, mesocotyl elongation decreased by approximately half.

Mesocotyl elongation in etiolated seedlings was also promoted by exogenously applied GA_3 (Fig. 2). When ABA and GA_3 were applied simultaneously, an additive effect on mesocotyl growth was observed (Fig. 2).

Endogenous Levels of ABA in Mesocotyl of Etiolated Seedlings Treated with ABA, Mefluidide, Mannitol, or PEG 6000

When etiolated seedlings were treated with ABA, mefluidide, mannitol, or PEG 6000, ABA levels in mesocotyl increased progressively in a chemical concentration-dependent manner (Fig. 3). As shown in Fig. 1, the most significant increases in the length of mesocotyl were observed at 0.31 μM for ABA, 3.12 μM for mefluidide, 1.56 mM for mannitol, and 1.25 mM for PEG (Fig. 1). Chemicals at these doses elicited twofold to threefold increases in endogenous levels of ABA (Fig. 3). The significant decreases in mesocotyl elongation could be observed at 20 μM for ABA, 50 μM for mefluidide, 100 mM for mannitol, and 20 mM for PEG (Fig. 1). Chemicals at these doses elicited fivefold to eightfold increases in endogenous levels of ABA (Fig. 3).

Discussion

The growth of mesocotyl in etiolated rice seedlings has been found to be regulated by many factors (Takahashi 1985). The number of cells in the mesocotyl increased when ABA was applied exogenously, and the length of mesocotyl cells increased when GA_3, indole-3-acetic acid (IAA), ethylene, or ABA was applied. Unlike rice, foxtail millet is a drought-resistant crop. In the field, the growing mesocotyl protects seedlings emerging from the soil and therefore enhances seedling survival. Little is known about the regulation of foxtail millet mesocotyl growth by plant hormones. Here we present evidence that ABA is involved in this process.

Our previous studies demonstrated that mesocotyl growth in a wild type foxtail millet cultivar and a GA-responding dwarf mutant (Chen et al. 1996b, 1998) was promoted by relatively low concentrations of GA_3 or ABA and that the response of mesocotyl in etiolated seedlings of the dwarf mutant was more sensitive than that of the wild type cultivar (Chen et al. 1997). In this study, mesocotyl growth in etiolated seedlings of a dwarf mutant CH84113 (Chen et al. 1996b, 1998) is surveyed. We show that ABA at relatively low concentrations can promote the growth of CH84113 mesocotyl, the length of which increases significantly (Fig. 1). It has been found that mefluidide, a synthetic plant growth regulator, is capable of triggering an increase in endogenous ABA levels (Zhang et al. 1986). Additionally, in many investigations of stress-induced ABA accumulation in leaves

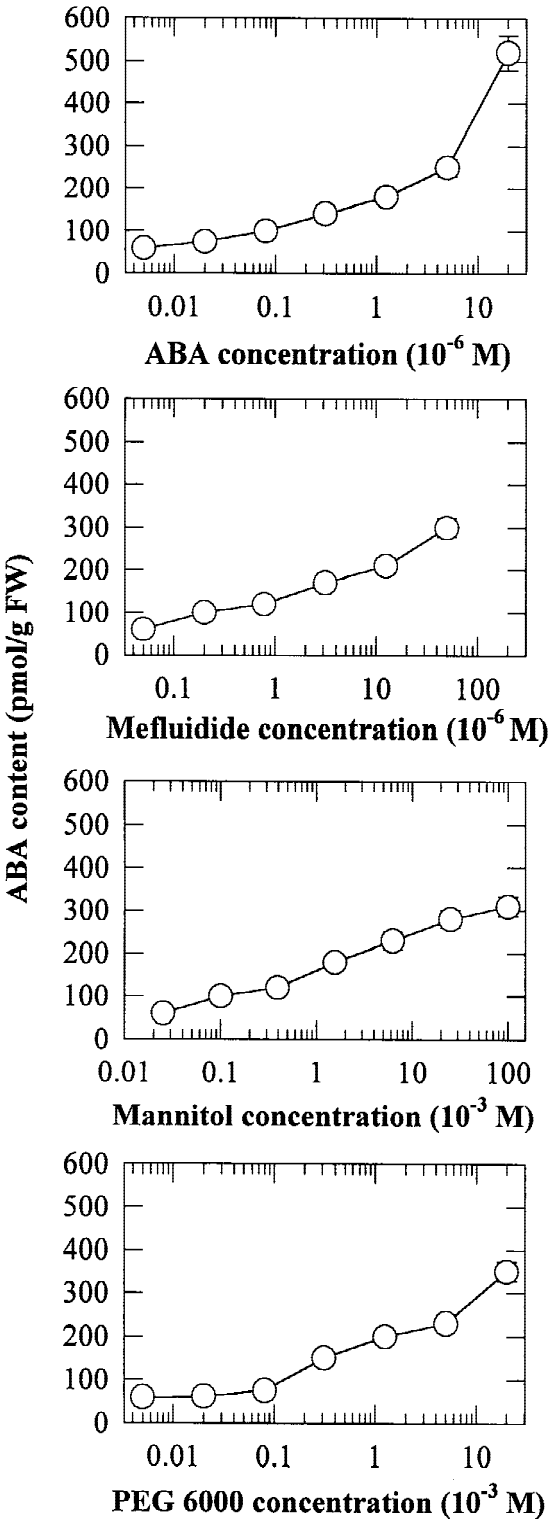

Fig. 3. Endogenous levels of ABA in mesocotyl of etiolated seedlings of CH84113 treated with various exogenous concentrations of ABA, mefluidide, mannitol, or PEG 6000. Data were collected 3 days after treatment. Shown are the averages ± S.E. of three replicates. The ABA content in untreated seedlings was 60.0 ± 4.5 pmol/g, fresh weight. *Vertical bars* denote S.E. when larger than symbols.

and roots, mannitol and PEG have been used as osmotica to regulate water potentials (Lin and Fu 1996, Xin et al. 1997). It is now well established that endogenous ABA levels increase considerably when plants are water stressed (Saab et al. 1990, Xin et al. 1997). As expected, when mefluidide, mannitol, or PEG 6000 was applied, endogenous levels of ABA in mesocotyl tissue increased progressively in a chemical concentration-dependent manner (Fig. 3). Effects of mefluidide, mannitol, and PEG 6000 on mesocotyl growth might be, therefore, mediated by these increased endogenous ABA levels.

It is interesting to note that although the leaf blade, leaf sheath, and shoot of the dwarf mutant CH84113 showed no response to exogenously applied GA_3 under light and as such it has been described as a non-GA-responding mutant (Chen et al. 1996b, 1998), the mesocotyl in the etiolated seedlings responded markedly to GA_3, indicating that the elongation of the mesocotyl may be regulated by a different system than leaf and shoot. Further, S-3307, an inhibitor of GA biosynthesis (Hedden 1990, Izumi et al. 1984), could strongly inhibit the growth of mesocotyls in foxtail millet etiolated seedlings (Fig. 2). When GA_3 and ABA were applied simultaneously, an additive effect was observed (Fig. 2). These results imply that ABA and GA might control different processes in the regulation of mesocotyl growth. Perhaps in a manner similar to rice, ABA may regulate mainly cell number, whereas GA regulates cell length in the mesocotyl (Takahashi 1985). We do not know exactly the effect of other plant hormones on the growth of foxtail millet mesocotyl. In vitro, GA_3 and ABA did not induce significant increase in the length of apical mesocotyl sections, the area of the most rapid cell elongation, whereas IAA did (data not shown). However, it has been found recently that auxin may have a less important role in elongation responses in dark-grown than in light-grown seedlings (Jensen et al. 1998). A more extensive survey of the effects of exogenous plant growth substances and the analysis of endogenous hormones is now under way.

Acknowledgments. This work was supported financially by the Special Foundation for Doctoral Study Center in National Universities, and the National Natural Science Foundation of the People's Republic of China. Thanks are due to Dr. Richard M. Napier (Department of Cell Physiology, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK) for a critical reading of the text and valuable suggestions.

References

Chandler PM, Robertson M (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 45:113–141

Chen JG, Du XM, Zhao HY, Zhou X (1996a) Fluctuation in levels of endogenous plant hormones in ovules of normal and mutant

cotton during flowering and their relation to fiber development. J Plant Growth Regul 15:173–177

Chen JG, Zhang YZ, Zhou X (1997) Regulation of foxtail millet mesocotyl growth by gibberellins and abscisic acid in etiolated seedlings. J Nanjing Agric Univ 20:13–17

Chen JG, Zhang YZ, Zhou X (1998) Characterization of gibberellin-responding and non-responding dwarf mutants in foxtail millet. Acta Agric Boreali-Sinica 13:46–52

Chen JG, Zhou X, Zhang YZ (1996b) Different response to millet mutants to GA_3 and their endogenous hormone analysis. J Nanjing Agric Univ 19:6–11

Davies PJ (1995) The plant hormones: Their nature, occurrence, and functions. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–12

Hedden P (1990) The action of plant growth retardants at the biochemical level. In: Pharis RP, Rood SB (eds) Plant growth substances 1988. Springer-Verlag, Heidelberg, pp 322–332

Izumi K, Yamaguchi I, Wada A, Oshio H, Takahashi N (1984) Effect of a new plant growth retardant (*E*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-s-ol (S-3307) on the growth and gibberellin content of rice plants. Plant Cell Physiol 25:611–617

Jacobsen JV, Gubler F, Chandler PM (1995) Gibberellin action in germinated cereal grains. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 246–271

Jensen PJ, Hangarter RP, Estelle M (1998) Auxin transport is required for hopocotyl elongation in light-grown but not dark-grown Arabidopsis. Plant Physiol 116:455–462

Lee TT, Zhou X (1996) Plant hormones and their immunoassays. Jiangsu Science and Technology Press, Nanjing, pp 158–206

Lin L, Fu JR (1996) Effect of osmoticum and fluridone on synthesis of storage proteins and LEA proteins of peanut embryos cultured in vitro. Acta Phytophysiol Sinica 22:368–372

Pengelly WL (1985) Validation of immunoassays. In: Bopp M (ed) Plant growth substances. Springer-Verlag, Berlin, pp 35–43

Rock CD, Quatrano RS (1995) The role of hormones during seed development. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 671–697

Saab IN, Sharp RE, Pritchard J, Voetberg GS (1990) Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol 93:1329–1336

Takahashi K (1985) Abscisic acid as an endogenous stimulator for rice mesocotyl growth. Chem Regul Plants 20:53–60

Walton DC, Li Y (1995) Abscisic acid biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 140–157

Weiler EW (1986) Plant hormone immunoassay based on monoclonal and polyclonal antibodies. In: Linskens HF, Jackson JF (eds) Immunology in plant sciences. Springer-Verlag, New York, pp 1–17

Xin ZY, Zhou X, Pilet PE (1997) Level changes of jasmonic, abscisic, and indole-3ylacetic acids in maize under desiccation stress. J Plant Physiol 151:120–124

Zhang CL, Li PH, Brenner ML (1986) Relationship between mefluidide treatment and abscisic acid metabolism in chilled corn leaves. Plant Physiol 81:699–701

Zhou X, Zheng ZF, Chen PY, Zhang D (1996) Preparation and application of monoclonal antibodies specific for abscisic acid methyl ester. Acta Phytophysiol Sinica 22:284–290