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EFFECT OF FLURIDONE ON PLANT DEVELOPMENT AND STRESS-INDUCED ABA ACCUMULATION IN *Vicia Faba* L. PLANTS

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Summary. The effect of fluridone on the growth of plants and drought stress-induced abscisic acid (ABA) accumulation were studied in mature leaf tissue of *Vicia faba* L. plants grown under different light intensity. Drought stress was induced by allowing the leaves to lose 12% of fresh weight. Treatment of *Vicia* plants (grown at high light intensity – 600 µmol.m^{-2}.s^{-1}) with 10 µmoles fluridone caused a considerable decrease in the rate of growth and bleaching of the plants. Fluridone fully blocked stress-induced ABA accumulation. Plants grown under low light intensity (40 µmol.m^{-2}.s^{-1}) had diminished level of ABA after impossition the leaves to dehydration. Fluridone treatment reduced the level of ABA in unstressed as well as dehydrated leaves. The accumulation of ABA in 21-day-old green *Vicia* plants was considerably reduced when they were exposed to dark periods (24 h, 48 h, and 72 h) just before the impossition of stress. Twenty four hours after dark treatment dehydration of these leaves resulted in a 3 fold decrease in stress-induced ABA accumulation. Seventy two hours after dark treatment the level of stress-induced ABA was approximately equal to the prestressed values. Fluridone-treated plants failed to accumulate ABA when water stressed. The presented results are discussed in term with the role of light and fluridone on stress-induced ABA accumulation.

Key words: abscisic acid, chloroplasts, drought stress, fluridone, *Vicia faba* L.

Abbreviations: ABA – abscisic acid, fluridone-(1-methyl-3-phenyl-5-[3-triflurомethyl(phenyl)]-4-(1H)-pyridinone)

Inroduction

Fluridone (1-methyl-3-phenyl-5-[3-triflurомethyl(phenyl)]-4-(1H)-pyridinone) is a herbicide whose mode of action at the molecular level has not been satisfactorily elucidated. It has been claimed that this compound is an inhibitor of the enzyme phytoene desaturase which converts phytoene to phytofluene (Bartels and Watson, 1978; Fong and Schiff, 1979). Similar mode of action has been reported for a pyridizanone herbicide norflurazon (Vaisberg and Schiff, 1976; Eder, 1979).

Treatment of plants, algae or cianobacteria with these herbicides results in accumulation of phytoene and concurrent bleaching of the organisms. Carotenoids and chlorophylls are essential constituents of photosynthetic membranes. Carotenoids are present in all photosynthetic organisms where they serve as accessory pigments for harvesting light and are also components of photosynthetic reaction center complexes (for review see Oelmuller, 1989). If carotenoid-free seedlings are kept under very low light, plastidogenesis is normal, and the accumulation of Chl *a* and *b*, many thylakoid membrane proteins and stromal enzymes is comparable to that in carotenoid-containing seedlings (Frosch et al., 1979; Oelmuller et al., 1986; Oelmuller, 1989). By growing barley seedlings in darkness Gamble and Mullet (1986) showed that fluridone inhibited carotenoid accumulation but did not alter plastid biogenesis and protein composition. Unfortunately, data on the ultrastructure of proplastids were not reported.

In addition, there exist evidences that carotenoids are the main precursors for ABA synthesis in plants (for review Zeevaart and Creelman, 1988).

If this is true then inhibiting carotenogenesis should also prevent the synthesis of ABA. This hypothesis has been supported by the works with carotenoid-deficient mutants or by blocking carotenogenesis with herbicides that do not respond to water stress by ABA accumulation (Quarrie and Lister, 1984; Gamble and Mullet, 1986; Stewart and Voetberg, 1987). Carotenoid-deficient mutants produce white seedlings due to photooxidation of chlorophyll in the absence of carotenoids. Non-mutant seeds treated with fluridone or norflurazon (Henson, 1984) also produce white seedlings due to the inhibition of carotenoid synthesis and the subsequent photooxidation of chlorophyll. In all these treatments plastidogenesis has been destroyed and plastid ribosomes were completely absent.

It has also been suggested that plastid ribozomes are essential for the synthesis of ABA in response to stress, and that the enzymes for ABA synthesis are encoded on nuclear genes (Quarrie and Lister, 1984).

In this study we have tried to distinguish the photobleaching effect of fluridone on plastid development and drought-induced ABA accumulation from the same effect of fluridone in non-photooxidative conditions.

Materials and Methods

Plant growth conditions, fluridone treatment and stress exposure

Seeds of *Vicia faba* L. were imbibed for 24 h in water containing 0 or 10 µmoles fluridone (a gift from Eli Lilly Research Laboratories). The hydrated seeds were grown in a Fafard M2 soilless medium (VJ Growers, Charlotte, NC, USA) and fertilizers were applied twice a week. Plants were cultured in a Percival growth cabinet (Environmental Growth Chambers, Chagrin Falls, OH, USA), day/night temperatures 25/20 °C, RH 60%, 16 h photoperiod and light intensity 600 $\mu mol.m^{-2}.s^{-1}$. The seedlings were watered daily with the respective solutions. Young, fully expanded bifoliate leaves of 3 to 4 weeks old plants were used in all experiments. One of two sister leaflets (control sample) was detached, quickly frozen in a liquid N_2 slurry and freeze-dried at -25 °C and 1.3 Pa for 48 h. The other leaflet (water-stressed sample) was dehydrated to 88% unstressed fresh weight. The water loss was induced by placing leaflets in a cool air stream for 10–15 minutes until a twelve per cent loss of fresh weight was obtained. After incubation in sealed plastic bags for 4 h in darkness at 23 °C, these stressed leaf samples were frozen and freeze-dried.

In another set of experiments the light intensity was reduced to 40 $\mu mol.m^{-2}.s^{-1}$ by shading with several layers of "Miracloth".

For dark treatment experiments, plants were grown for three weeks under the standard growth conditions and then they were exposed in continuous darkness. Samples were taken 24 h, 48 h, and 72 h after dark treatment.

ABA analysis

ABA was extracted from leaf tissue samples (200–250 µg dry mass) with 80% v/v aqueous and then 100% methanol + 100 $mg.L^{-1}$ butylated hydroxy toluene (1 µg of tissue dry weight per 1 µl of extract) for 24 and 6 h, respectively, at 4 °C in darkness. After drying the combined extracts under N_2 the residue was dissolved in 50 µl methanol and diluted with 200 µl Tris-buffered saline (50 mM tris-HCl, pH 7.8, containing 150 mM NaCl and 1 mM $MgCl_2$).

ABA was estimated by enzyme-amplified ELISA as described by Zhang et al. (1991).

Results

Growth characteristics of *Vicia faba* L. in the presence of fluridone. Leaf anatomy

Tratment of *Vicia faba* L. plants with 10 µmoles fluridone caused a strong reduction in the rate of growth. The leaves of treated plants were small, fully developed and completely white. In addition, 2 to 3 shoots were observed close to the main stem, results often associated with vivipary and correlated with a low ABA level (Fig. 1).

Fig. 1. Effect of fluridone (10 µmoles) on the growth and development of *Vicia faba* L. plants. A – control plans; B – plants grown in the presence of 10 µmol fluridone. Light intensity 600 µmol m^{-2} s^{-1}.

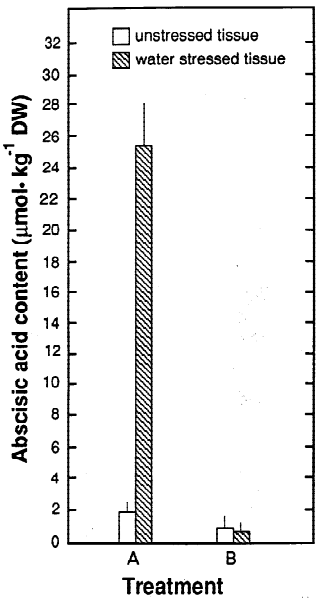

Fig. 2. ABA content of control and fluridone-treated (10 µmoles) *Vicia faba* L. plants grown at high light intensity (600 µmol.m^{-2}.s^{-1}). Unstressed leaflets (open columns) were quenched immediately in liquid N_2. Stressed leaflets (shaded columns) were dehydrated to 88% fresh weight and then incubated in darkness for 4 h before quenching. A – control plants; B – plants grown in the presence of fluridone. Values are means ± SD (n = 4).

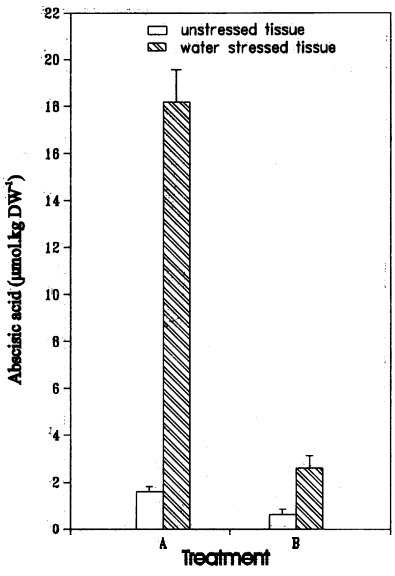

Fig. 3. ABA content of control and fluridone-treated *Vicia faba* L. plants that were grown at low light intensity (40 µmol.m^{-2}.s^{-1}). Unstressed leaflets- open columns; stressed leaflets- shaded columns. A – control plants; B – plants grown in the presence of fluridone. Values are means ± SD (n = 2).

Effect of light intensity on stress-induced ABA accumulation

ABA levels of control and dehydrated *Vicia faba* L. leaves which had been grown at 600 µmol.m^{-2}.s^{-1} light intensity and treated with 10 µmoles fluridone are shown in Fig. 2. The ABA level of control plants was low (1.9 µmol.kg^{-1} DW) but increased more than 13 fold after dehydration of the leaves followed by a 4 h incubation. Detectible levels of ABA were found in leaves from plants grown on 10 µmoles fluridone. The data presented here showed that plants grown in the presence of fluridone and high light intensity had diminished levels of ABA in unstressed as well as dehydrated leaves.

Growing plants at low irradiance (40 µmol.m^{-2}.s^{-1}) reduced water stress-induced ABA accumulation. Fluridone treatment diminished the level of ABA in unstressed as well as dehydrated leaves (Fig. 3).

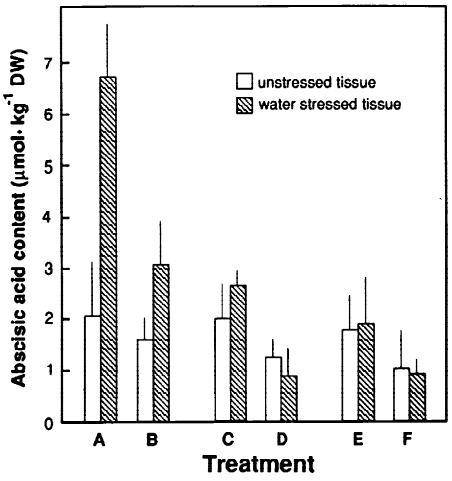

Fig. 4. ABA content of control and fluridone treated green *Vicia faba* L. leaflets after exposure to continuous darkness for 24, 48, and 72 h. Unstressed leaflets (open columns), stressed leaflets (shaded columns). A – untreated control after 24 h in the dark; B – 10 µmoles fluridone-treated plants for 24 h in the dark; C – untreated control after 48 h in the dark; D – fluridone-treated plants for 48 h in the dark; E – untreated control after 72 h in the dark; F – fluridone-treated plants for 72 h in the dark. Values are means ± SD (n = 4)

Further experiments were performed to determine whether the integrity of chloroplast structure is required for ABA accumulation after imposition to water stress or lack of carotenoids after fluridone treatment leads to inhibition of ABA synthesis. Plants were routinely grown at light intensity about 600 µmol.m^{-2}.s^{-1} for 21 days and at the end of the normal 10 h dark period they were exposed to a continuous darkness and treated with 10 µmoles fluridone for 24 h, 48 h, and 72 h. The levels of ABA from control and fluridone treated plants were measured in turgid and stressed leaves (4 h after imposition of water stress). ABA accumulation in control leaves in response to water stress was markedly inhibited in the 24 dark treated plants (Fig. 4). The stress-induced ABA accumulation dropped from 25 µmol.kg^{-1} DW in light grown plants (Fig. 2) to 6.7 µmol.kg^{-1} DW after 24 h dark treatment (Fig. 4A). A prolonged dark treatment (48 h and 72 h) reduced the levels of ABA to 2.7 µmol.kg^{-1} DW (Fig. 4C) and 1.75 µmol.kg^{-1} DW (Fig. 4E), respectively, which values are a little higher to the pre-stressed ABA levels. Fluridone also inhibited the stress-induced ABA accumulation but the effect was less expressed than that to high light (compare Fig. 2 and Fig. 4).

Discussion

It has been reported that carotenoid biosynthesis inhibitors fluridone and norflurazon lead to decrease in photosynthesis (Lem and Williams, 1981), ribosome number per plastid and plastid rRNA synthesis (Bartles and Watson, 1978; Reib et al., 1983), chlorophyll per leaf (Vaisberg and Schiff, 1976), and also alter lipid composition (Lem and Williams, 1981).

Furthermore, the most striking visible effect of herbicide treatment is the albino appearance of foliar tissue. The degree of bleaching varies widely depending on the intensity of illumination under which the plants are grown, herbicide concentration used, the mode of treatment given and the nature of the plant species used (St John, 1976; Stewart and Voetberg, 1987). Pigment bleaching, however, is a common secondary effect of herbicides (Matringe et al., 1989a, 1989b), and thus conclusions about whether it is the primary site of action of herbicide should be treated with caution.

It is difficult to extrapolate an effect from one plant to another, especially since plants vary in their sensitivity to fluridone and large variation of concentrations have been used. For monocot barley and maize Gamble and Mullet (1986) and Stewart and Voetberg (1987) used 0.1 mM fluridone for barley and 100 $mg.L^{-1}$ (approximately 0.3 mM) for maize (Moore and Smith, 1984) without any visible damages of the plants, exept the observed leaf tissue bleaching. Resently Xu and Bewley (1995) showed that treatment of *Medicago sativa* L. seeds with 0.01% fluridone did not change the morphology except the colour of seeds and did not lead to viviparous germination. In our experiments with dicot *Vicia faba* treatment with 0.1 mM fluridone prevented seed germination and even after application of 10 fold lower concentration (0.01 mM) the rate of growth was severely restricted (Fig.1). Our attempts to grow *Vicia* plants in darkness were not successful because the plants did not develop normal well expanded etiolated leaves. Treatment of plants with 10 µmoles fluridone in darkness resulted in a very low rate of growth and also leaves became completely white (data not presented).

Another observation made in our study was that the leaves of light grown and fluridone-treated plants did not contain chloroplasts but only small chlorophyll-free rudiments whose internal structure had almost disappeared (data not presented).

These results allowed us to test the level of stress-induced ABA accumulation in the presence of photooxidative damage for the chloroplasts (light grown plants), and in the absence of plastid photodestruction (dark-treated plants). ABA level of control light-grown plants increased more than 13 fold after dehydration of plants followed a 4 h incubation (Fig.3). These data are consistent with these previously reported by Harris and Outlaw (1991) and Zhang et al. (1991) for the same plant species. Plants grown in the presence of 10 µmoles fluridone under high light had diminished levels of ABA in unstressed as well as dehydrated leaves. They had no capacity to accumulate ABA after dehydration.

Limited data suggest that light environment to which plants are exposed prior or during stress treatment may be an important factor influencing ABA production (Henson, 1983, 1984). It was found that the level of ABA in intact unstressed plants, and amounts induced by water stress were greater in light than in dark grown plants (Simpson and Saunders, 1972; Tietz, 1974), and stress-induced ABA accumulation may be favored by higher rather than lower light intensities (Rajagopal and Andersen, 1980; Moore et al., 1985).

Our data presented in Fig. 4 show that low light intensity decreased the level of stress-induced ABA accumulation. Fluridone-treated plants grown under the same light intensity were not capable to accumulate ABA after water stress imposition. Unfortunately, we have no data on the effect of fluridone on chloroplast ultrastructure in treated plants. We could indirectly assume that the plastid structure had been damaged because even under that low light intensity 10 µmoles fluridone caused photobleaching of leaves.

The amount of ABA was considerably reduced when the green plants were exposed to 24 h dark period just before the imposition of stress, and after a 72 h dark period the level of stress-induced ABA reached that of the prestressed.

One explanation for these results is that only leaves which lack precursor carotenoids (light grown plants) are unable to make ABA. As the lack of carotenoids destroyed the chloroplast ultrastructure it is very difficult to answer which of the two factors is more important for ABA accumulation. This would imply that structurally intact and/or functionally active plastids are required for drought stress to elicit a rise in ABA. We also do not rule out the possibility that during dark treatment an important reason for the lowered level of stress-induced ABA could be the reduced synthesis of NADPH and ATP. Only after prolonged dark treatment fluridone caused a variety of anomalies and the lack of ABA accumulation is an indirect result of plastid disfunction.

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