ACTA BIOL. SZEGED. 41, pp. 93-95 (1995/1996)

Summary of thesis submitted for the degree of Candidate of Science

THE ROLE OF ETHYLENE IN THE GROWTH AND DEVELOPMENT OF BEAN SEEDLINGS TREATED WITH THE GROWTH RETARDANT PACLOBUTRAZOL

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Introduction

Plant growth retardants are synthetic growth regulators which reduce the height of plants by inhibiting cell division in the subapical meristems and by reducing the cell elongation in the stem without exerting a substantial effect on the numbers of internodes and leaves.

Plant growth retardants are frequently applied in agricultural technology and horticulture to improve the lodging resistance of cereals, to reduce the height of orchard trees and to save the trimming costs of bushes and hedges. Moreover, growth retardants improve the resistance of plants to abiotic and biotic stresses.

Paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol), a triazole retardant, has been shown to inhibit gibberellin biosynthesis by blocking the oxidation of ent-kaurene to ent-kaurenoic acid. This reaction is catalyzed by cytochrome P-450-dependent monooxygenases, which are inactivated by paclobutrazol.

Plant growth retardants result in a new hormonal balance in treated plants: the concentrations of cytokinins increase as compared to the levels of abscisic acid and ethylene. The ethylene release of tissues decreases in heterotrophic cell cultures, leaf discs or stem segments after paclobutrazol treatment, and it was concluded that ethylene production is inhibited by paclobutrazol as a result of the altered composition of the membrane lipids of retardant-treated cells. These changes in the membrane properties influence the conversion to ethylene of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor, by decreasing the activity of the membrane-bound ACC oxidase.

We found, however, that bean seedlings treated with paclobutrazol exhibited morphological changes which are evoked or controlled by the plant hormone ethylene, such as

- the increased lateral expansion of the hypocotyl bases of light-grown seedlings,
- the decreased expansion of the leaves,
- modifications in the movement and position of the primary leaves,
- control of adventitious root initiation on cuttings.

The aim of this work was to examine the role of ethylene in the development of these morphological changes in paclobutrazol-treated bean seedlings.

Materials and Methods

Seeds of bean (*Phaseolus vulgaris* cv. Juliska) were soaked in 4.25×10^{-7} - 1.7×10^{-5} M paclobutrazol (ICI Agrochemicals, Bracknell; UK) and they were grown under controlled conditions in a growth chamber.

The ethylene production of the tissues was determined with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and an activated alumina column. The ACC and malonyl-ACC contents of the tissues were measured after chemical conversion to ethylene by the method of LIZADA and YANG. The vacuum extraction of ethylene was carried out by the method of SCHIERLE and SCHWARK.

The indole-3-acetic acid contents of samples were measured spectrofluorimetrically after conversion to indole-α-pyrone, with a Perkin-Elmer spectrofluorimeter. Enzyme activities were determined with a UVIKON 930 spectrophotometer.

Results

The ethylene production by the organs of paclobutrazol-treated bean plants may be lower or higher than that of the control, or equal to that of the untreated tissues.

In etiolated plants, the ethylene production by the elongation and basal zones of the treated hypocotyls was not lower than in the control, but in the light-grown bean the retardant resulted in a significant reduction in ethylene release by the elongation zone of the hypocotyls. The basal zone of the treated hypocotyls in this latter case exhibited a significant increase in ethylene production as compared to the apical part.

The ethylene production by the blades of the primary leaves were lower in retardant-treated plants in the light, but after a sharp increase at a light/dark transition, this difference was equalized.

The laminar pulvinus and the petiole of the paclobutrazol-treated primary leaves evolved significantly more ethylene than the controls.

During the induction of adventitious roots on the primary leaf cuttings, the ethylene production of the root-forming tissues was not lower in the treated cuttings than in the control, but the kinetics was different.

In those cases where we found an increased ethylene production in the treated tissues, the concentration of ACC, the precursor of ethylene in plant cells, was also higher, which was accompanied by a decreased malonylation.

In the hypocotyls of light-grown seedlings, the retardant treatment induced a lateral swelling in the basal part, without increasing the cell number in the radial direction. This coincided with the increased ethylene production and indole-3-acetic acid content of the tissues. In etiolated plants, paclobutrazol treatment resulted in a shift in the accumulation of ethylene and ACC towards the base of the hypocotyls, identically to the action of white light, which exerted a similar effect in both control and retardant-treated hypocotyls. This means that in the dark paclobutrazol acted similarly to white light. This phenomenon may provide a common basis for the growth-retarding effect of white light and paclobutrazol.

The cell number in the primary leaves was not affected by paclobutrazol. In the leaf blades, the 200% increase in vacuum-extractable ethylene at the light/dark transition may be regarded as a "natural ethylene treatment" which modifies the expansion of the treated leaves. The effect of ethylene on the leaf expansion may be mediated by peroxidases, which exhibited an increased activity in treated leaves, both in the soluble fraction and in the fractions bound ionically and covalently to the cell wall. Peroxidases secreted into the apoplast catalyze the formation of intermolecular bonds between cell wall polymers leading to the cessation of cell expansion.

The primary leaves of *Phaseolus* display a nyctinastic movement, which is maintained by the cyclic changes in the turgor pressure of the motor cells in the flexor and extensor regions of the pulvinus. The cells of the laminar pulvinus released much more ethylene and contained more ACC in treated plants, which made the night position of the leaf blades permanent by decreasing the auxin activity of the tissues. The indole-3-acetic acid content of the petioles was reduced as compared to the control. The shortage of auxin contributes to the maintenance of the night position of the leaves because the electrogenic pumps of the motor cells are activated by indole-3-acetic acid.

The inhibitors of ethylene biosynthesis or action, aminooxyacetic acid and Co^{2+} ion or 2,5-norbornadiene and Ag^{+} ion, respectively, effectively inhibited the development of these morphological phenomena in treated plants, the lateral expansion of the light-grown hypocotyls at the base, and the sleeping position of the primary leaves in the light, and partially reversed the inhibition of primary leaf expansion. These morphological changes can be evoked in control plants by the ethylene generator Ethrel and the precursor ACC which suggests that ethylene is involved in the control of these processes.

Paclobutrazol is frequently used to improve the adventitious root formation on cuttings. In contrast, it was found that the primary leaf cuttings of paclobutrazol-treated bean plants rooted very poorly. The time course of ethylene production during the rooting process revealed that the ethylene production of the root-forming petioles was not lower in the treated cuttings than in the control. The rooting capacity of these cuttings could be restored by applying abscisic acid or Ethrel exogenously on the blades. Treatment with 5 μM abscisic acid resulted in an increased ethylene production and ACC content of the root-forming petioles 48 hours after treatment. The effect of abscisic acid could be reversed by 10 μM $CoCl_2$ in the rooting solution, which decreased the ethylene production of the tissues by about 50%. It was concluded that the inhibition of adventitious root formation in paclobutrazol-treated primary leaf cuttings could be developed by a relative ethylene deficiency.

On the basis of these results, it was found that the ethylene production by the tissues of paclobutrazol-treated bean plants can be lower than, higher than or equal to that of the control. The ethylene production determines the morphology of the organs, while the effect of ethylene depends not only on the absolute concentration, but also on the physiological and hormonal status of the tissues.

This work afforded new information both on the physiological effects of paclobutrazol and on the role of ethylene in these developmental processes. Data on the control of leaf development and adventitious root formation by paclobutrazol may provide direct information for users of plant growth regulators.