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CHANGES IN PEROXIDASE ACTIVITY AS AFFECTED BY GIBBERELLIC ACID AND CYCOCEL IN CUCUMBER SEEDLINGS

S. SAROOP, V.S. THAKER, P.P. VAISHNAV AND Y.D. SINGH

Department of Biosciences, Saurashtra University, Rajkot-360 005, India

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Abstract

Light and dark grown cucumber seedlings treated with GA_3 and CCC were analysed for growth as well as peroxidase activity in cytoplasmic and salt-extracted ionically wall bound fractions with a view to test the antigibberellin property of CCC. Root elongation was inhibited by both GA and CCC under both the conditions of light and dark, whereas hypocotyl growth was promoted by GA and inhibited by CCC. Peroxidase activity in dark showed inverse relationship with growth promotion. It is suggested that GA induced effect(s) on peroxidase activity is tissue specific and not always of a depressive nature. Further, root peroxidase patterns in GA and CCC indicated that CCC does not always act as an antigibberellin and may have a more direct control on peroxidase synthesis.
Key words: cucumber seedlings, CCC, GA_3, peroxidase activity

Introduction

Cycocel (CCC), a growth retardant has been commonly used to understand the mode of gibberellin action (see SEMBDNER et al., 1980). This is mainly due to the early demonstration (LOCKHART, 1962) that CCC interacts with GA_3 in a specific, competitive manner, and saturating dose of GA_3 can easily overcome the inhibitory effect. CCC inhibits the activity of kaurene synthetase — a key enzyme in GA biosynthesis (FROST and WEST, 1977) and leads to accumulation of geranyl-geranyl pyrophosphate (BARNES et al., 1969). It has also been proposed that CCC acts as an "antigibberellin" (LANG, 1970). However, concentration dependent (REID and CROZIER, 1972) and tissue dependent (GASPAR et al., 1971) controversial reports of CCC cast doubts on its "antigibberellin property". If CCC is a specific antigibberellin it should typically reverse GA action. Although the precise mechanism of GA action is still unknown, it has been proposed that it acts through auxin for elongation growth (PHILLIPS, 1969). Supportingly, GA application resulted in a marked increase in endogenous auxin level (KURAISHI and MUIR, 1963; LAW and HAMILTON, 1984); either by increase in its enzymic syntheis or suppression of its peroxidative breakdown. GA induced inhibition of peroxidase has been shown in a number of cases (FRY, 1979; 1980). Likewise, peroxidase participation in growth regulation and its inverse relation with growth is well documented (see LAMPORT,

1980; LAMPORT and CATT, 1981). Further, that wall bound peroxidase acts as a wall rigidfying factor leading to cessation of elongation growth (FRY, 1979) has also been proposed. It was therefore thought interesting to test the antigibberellin property of CCC using peroxidase activity as a metabolic probe.

Cucumber seedlings were treated with CCC and GA_3 and cytoplasmic and wall bound peroxidase activities were determined during early seedling growth.

Materials and Methods

Seeds of cucumber *(Cucumis sativus* L. Cv. 'Long green'), obtained from Pocha Seeds Company, Poona, India, were surface sterilized for 10 min using 0,1% mercuric chloride ($HgCl_2$). They were then thoroughly washed, rinsed with distilled water and placed over a moistoned filter paper (Whatman 1) in Petri dishes (15 cms). The Petri dishes were kept in a BOD incubator (25 ± 2°C) for 36 h to allow germination. Uniformly germinated seeds were transferred under green safe lamp to other Petri dishes (9 cm ∅) containing a filter paper with 5 ml of (1) Distilled Water (DW), (2) Gibberellic acid (GA_3, 100 mg/l) or, (3) 2-chloroethyl-trimethylammonium-chloride (CCC, 500 mg/l). Two such sets were prepared and incubated in dark and light. Samples were taken at every 24 hourly interval up to 96 h. Growth in terms of length of root and hypocotyl from 20 seedlings was measured and average was taken for calculations of percent over control.

Enzyme extraction

Samples from each treatment were dissected into root and hypocotyl and the organs were chilled for one hour at 0°C. Known amount of organs were homogenized in 5 ml of ice-cold acetate buffer (0.02M, pH 5.0). The homogenate was centrifuged at 12000 g for 10 min and the supernatant served as the source of cytoplasmic peroxidase activity. The pellet was washed with the same buffer till no peroxidase activity could be detected in the washings. The pellet was then incubated for 1 h with 1M sodium chloride to release the ionically wall bound enzyme. It was again centrifuged at 12000 g for 10 min and the supernatant served as wall bound enzyme.

Enzyme assay

The assay method of MAEHLY (1954) was followed and peroxidase activity was measured by recording the change in absorbance at 470 nm (ΔA_{470}) due to the oxidation of guaiacol in presence of H_2O_2. The activity was calculated for ΔA_{470} min^{-1} gm Fr $wt.^{-1}$ and expressed as percentage over DW control values.

Results and discussion

Results presented in Fig. 1 clearly show that in light and dark grown cucumber seedlings GA treatment remarkably inhibited root growth after an initial promotion, whereas hypocotyl growth was significantly promoted. The promotion in hypocotyl growth was highest after 24 and 48 hours of GA application in light and

dark respectively which decreased thereafter. Growth promotion in hypocotyl is accompanied by peroxidase suppression. On the other hand, growth inhibition in roots is accompanied by increase in peroxidase activity. Thus inverse relationships between percent control peroxidase activity and tissue growth is evident; only except in the light exposed hypocotyl. Such inverse relationships between peroxidase activity and growth indicating its active participation in growth reactions are commonly observed. For example reduced growth in dwarf plants is characterized by higher levels of peroxidase activity than the tall plants (SHERTZ et al., 1971). Likewise, while studying peroxidase ontogeny in dwarf pea stems, MCCUNE and GALSTON (1959) reported that GA application to young internodes results in a persistant depression in peroxidase activity and thereby promote the growth of internodes. The physiological significance ascribed to this enzyme is to control the biological activity of IAA in plants (see SEMBDNER et al., 1980) and in fact many of the GA induced growth promotions are explained on the basis of the earlier proposal (PHILLIPS, 1969) that GA affects indirectly via auxin. GA induced increase in auxin levels have been reported in several cases (KURAISHI and MUIR, 1963). Recently, LAW and HAMILTON (1984) using HPLC with electrochemical detection technique confirmed GA induced auxin promotion in dwarf peas.

The absence of a clear inverse relationship between percent control growth and peroxidase activity in light exposed hypocotyls may be due to interference of many other compounds in light. A number of phenolics interfere with peroxidase activity and auxin biosynthesis (KEFELI et al., 1974). According to KEFELI and KUTACEK (1977) phenolic compounds interfere in general, non-specific way with overall metabolic reactions involved in growth processes like photosynthetic and oxidative phosphorylation, biosynthesis of nucleic acids, proteins, etc. Besides phenolics another important factor is the hormonal balance as suggested by LALORAYA et al., (1970) that light causes a switchover from a gibberellin dominant growth and metabolism to the cytokinin-type of responses and that cytokinins do not antagonize gibberellin effects. Thus light triggers a complex set of parameters and they all may be collectively effecting peroxidase levels in hypocotyls. Gibberellins were considered to have little or no effect on root growth (CLELAND, 1969), however, our results show inhibitory effects. Similar results have been reported in a number of cases (SEVNSSON, 1972, LOY and LIU, 1974, BHATT et al., 1976).

Results on CCC induced changes in growth and peroxidase activity in light and dark grown cucumber seedlings are presented in Fig. 2. Like the GA treatment root growth is inhibited at later stages by CCC application but, unlike GA, it also remarkably inhibited the hypocotyl growth under dark and light conditions; the inhibitory effect increased with time. Most of the physiological effect of growth retardants are generally considered to be due to their inhibiting effects on GA biosynthesis (CATHEY 1964, LANG 1970) but our results do not support this conclusion since both GA and CCC inhibit root growth severely at later stages and promote peroxidase activity considerably (Figs. 1, 2). Further, although CCC induced inhibition in growth is accompanied by tremendous increase in peroxidase activity in root and hypocotyl, the trends between peroxidase and growth were

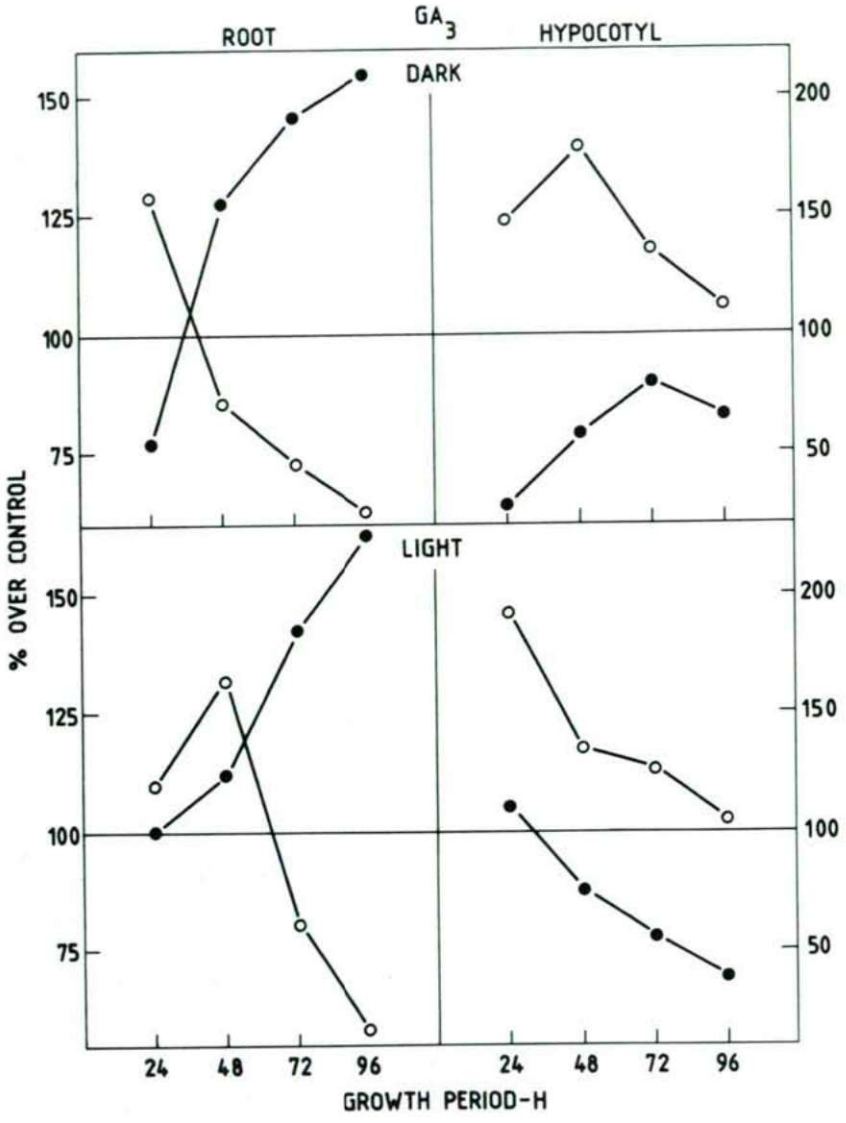

Fig 1. Effect of GA_3 on growth (α) and cytoplasmic peroxidase activity (β) in light and dark grown cucumber seedlings.

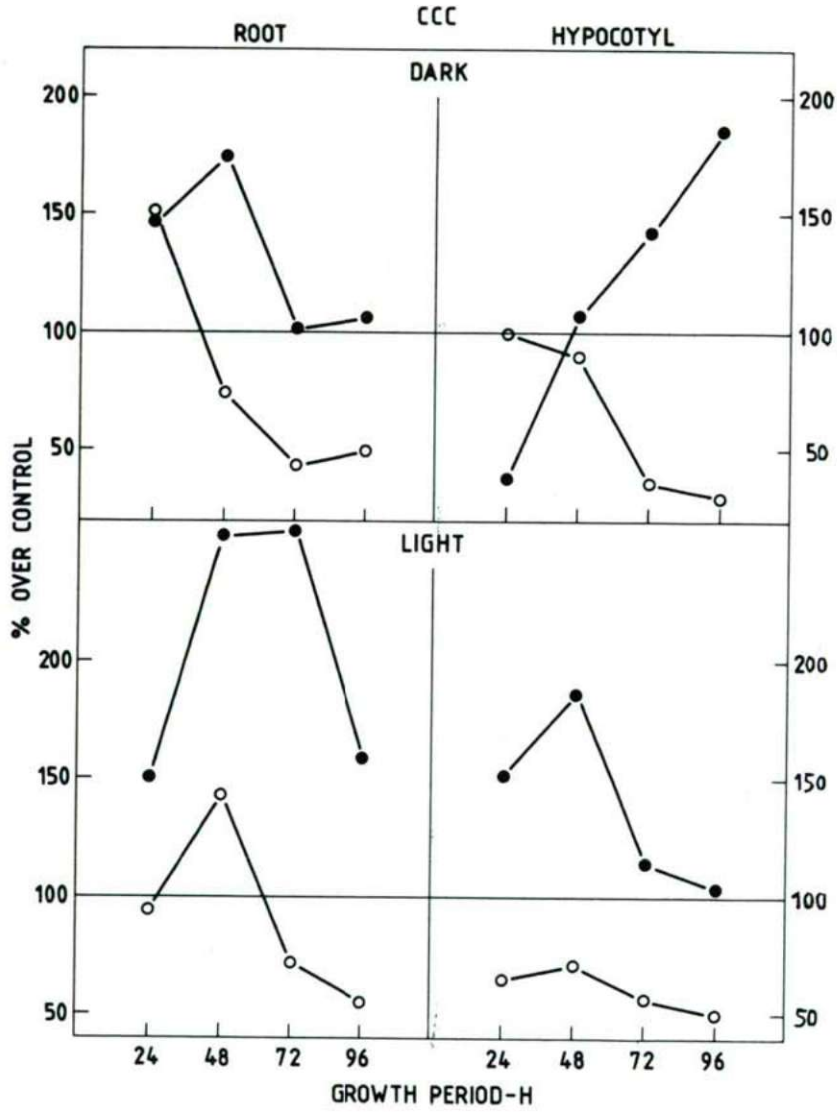

Fig 2. Effect of CCC on growth (α) and cytoplasmic peroxidase activity (β) in light and dark grown cucumber seedlings.

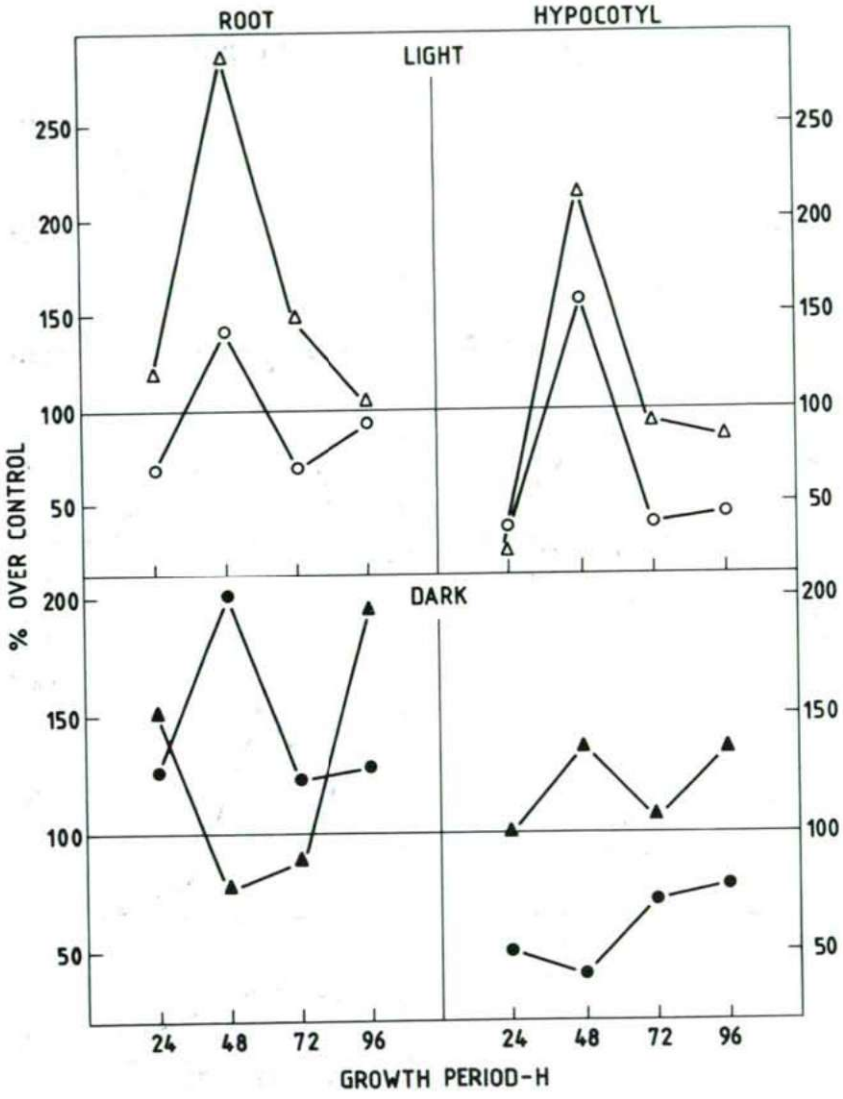

Fig 3. Salt-extractable ionically wall-bound peroxidase activity in GA_1 (α,β) and CCC (ε,ϕ) treated cucumber seedlings; blank and filled symbols represent light and dark respectively.

parallel except in the dark exposed hypocotyl. Thus it appears that CCC acted as antigibberellin only in hypocotyl and not in root. GRAEBE (1968) suggested that only high concentration of growth retardants may reflect an inhibition of GA biosynthesis. In the present work a fairly high concentration (500 mg/l) of CCC was used but even in such situations it did not show antigibberellin activity. Thus CCC may have its own mode of action as suggested CLELAND (1965).

The salt-extracted wall bound peroxidase activity in light grown GA and CCC treated seedlings (Fig 3), showed parallel trends in root as well as hypocotyl. However, under dark conditions GA and CCC possessed inverse trends showing the antigibberellin property of CCC. It is interesting to note that in dark GA promoted wall bound peroxidase in root whereas inhibited it in hypocotyl. Thus GA's effect on peroxidase activity appears to be tissue specific. On the other hand CCC promoted wall bound peroxidase in dark hypocotyl also. Cessation of elongation growth has been well correlated with wall bound peroxidase in diverse systems (RAMA RAO et al., 1982; FRY, 1979).

It is postulated that peroxidase restricts growth in two ways (a) covalently by catalysing the conversion of ferruloyl side chains into diferuloyl cross links and (b) non-covalently by catalysing the conversion of soluble phenolics into hydrophobic biphenyls, polymers or quinones, any of which could protect wall polysaccharides against the attack of wall glucanases.

This work leads us to conclude the following that (I) GA effects on cytoplasmic and wall bound peroxidase are tissue specific, (II) the antigibberellin property of CCC is dependent upon tissue and light conditions, (III) CCC may have its own effects other than GA biosynthesis inhibition, and (IV) peroxidase activity in cytoplasm may be playing an important role in controlling cellular auxin levels while that associated with wall fraction may be responsible for wall rigidification through its action on wall phenolics.

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