BIOLOGIA PLANTARUM (PRAHA)

28 (5) : 361–369, 1986

GA, ABA, Phenol Interaction in the Control of Growth: Phenolic Compounds as Effective Modulators of GA-ABA Interaction in Radish Seedlings*

S. D. RAY

College of Pharmacy, Rutgers State University of New Jersey,
Piscataway, N. J. 08854, U.S.A.

Abstract. Abscisic acid, a potent growth inhibitor inhibits hypocotyl growth of *Raphanus sativus* seedlings. Phenolic compounds, *viz.*, trans-cinnamic acid, chlorogenic acid, ferulic acid, salicylic acid, tannic acid and quercetin when applied with ABA, antagonize ABA action and restore normal seedling growth.

Gibberellic acid promotes hypocotyl growth and on combined application with ABA, the ratio of their concentrations determines the course of the resultant growth. This interaction can be modulated by phenolic compounds. Phenolic compounds in low concentrations when present together with GA and ABA, favour GA-induced growth by antagonizing the inhibitory influence of ABA.

The inhibitory action of abscisic acid on a wide range of growth processes is so far known to be reversed only by growth promoting hormones, *viz.*, IAA, GA and cytokinins. Antagonistic action of phenolic compounds towards ABA, and increasing the action of GA when present together with GA and ABA, establishes a dual role to this class of compounds; balancing the effect of both growth promoting and growth inhibiting hormones.

Abscisic acid inhibits growth of the hypocotyl in light-grown seedlings of *Amaranthus* (RAY *et al.* 1980), lettuce (DURLEY *et al.* 1976), radish (GURUPRASAD and LALORAYA 1980), dark-grown seedlings of *Trigonella* (MEGHA and LALORAYA 1977) and GA-induced hypocotyl growth in the seedlings of lettuce (SANKHLA and SANKHLA 1968, DURLEY *et al.* 1976).

Another class of compounds which have been shown to inhibit hypocotyl growth either in light or darkness are the phenolic compounds (RASMUSSEN and EINHELLIG 1977, MEGHA and LALORAYA 1978, LODHI 1979) which also inhibit GA-induced growth (CORCORAN 1976).

Thus, it appears that abscisic acid and phenolic compounds seem to have apparently similar action on seedling growth. However, on simultaneous application of the two compounds, their individual inhibitive nature disappear and instead of having an additive effect phenolic compounds counteracted the inhibitory action (RAY *et al.* 1983) and promotive action of ABA (EKATERINA and POGONCHEVA 1976, APTE and LALORAYA 1982).

Received November 20, 1984; accepted June 26, 1985

* Part I.

Phytohormones act in coordinating plant growth and development through interconnecting reactions that are at present poorly understood. Abscisic acid and gibberellins tend to bring about opposite physiological effects though the exact mechanism is not clear to date. We present results here to show that phenolic compounds in low concentrations show an antagonistic reaction towards ABA and when present together with GA and ABA, eliminate ABA action and favour GA-induced growth. In our earlier publication we have already demonstrated the additive effect of phenolic compounds on Kn-stimulated betacyanin synthesis. Therefore, in the present studies, the behaviour of phenolic compounds towards ABA has been analysed in the presence of a dominant hypocotyl growth inducer *e.g.* GA. The involvement of ABA (BORKOVEC *et al.* 1984) and phenolic compounds (KEFELI and KUTÁČEK 1976) in plant growth reactions has already been established and this antagonistic action of phenolic compounds on ABA-suppressed seedling growth would establish that they have a new regulatory role in the process.

MATERIAL AND METHODS

Seeds of *Raphanus sativus* L. (cv. Crimson French Breakfast, obtained from Sutton seeds Ltd., Calcutta) were germinated in the dark (25 ± 2 °C) for about 30 h on moist filter papers in 15 cm Petri dishes. Twenty germinated seeds with 4—5 mm radicle were transferred to 9 cm Petri dishes with filter paper containing either phosphate buffer or solution of GA_3 (gibberellic acid), ABA (abscisic acid), GA_3 + ABA, ABA + phenol, and GA + ABA + phenol. Final concentrations of the compounds in the mixtures are indicated in the figures and all the solutions were buffered. Then the Petri dishes were transferred either to darkness (25 ± 2 °C) or under continuous white light for further growth upto 72 h. Growth of the seedlings was measured at the end of 72 h dark/light (60 W m^{-2} at Petri dish level obtained through 40 W Philips white cool-daylight fluorescent tubes plus 100 W Philips incandescent bulbs, 25 ± 2 °C) period. ABA (± cis trans 95 %), GA_3 (90 %), trans-cinnamic acid, chlorogenic acid (Sigma Chemical Co., USA), ferulic acid, salicylic acid, tannic acid and quercetin (Fluka, Switzerland) were dissolved in either hot or cold buffer (0.02 M, pH 6.5, Na-phosphate) and the pH adjusted with diluted HCl or NaOH. The use of either buffer or distilled water did not affect the experimental results to any extent (RAY 1983).

RESULTS

ABA inhibited hypocotyl growth of both light and dark-grown radish seedlings and the inhibition shows a concentration response (Fig. 1). Inhibitions are comparatively less at these concentrations in light. ABA action seems to be more potent in the dark (Fig. 1).

To observe the interaction in this species, phenolic compounds were applied (10^{-7} to 10^{-4} M) with 4×10^{-6} M ABA in the dark (Fig. 2, a—f) and 10^{-5} M ABA in light (Fig. 3, a—f). To avoid any possible synthesis of phenols or destruction of ABA, all experiments were done in complete darkness first and then in light. Of the six phenolics tested in both light and dark, all of them alleviate ABA-induced inhibition of hypocotyl growth to various degrees. Compounds used show no variation in their effect in antagonizing

ABA action, they vary only quantitatively. Reversal is evident irrespective of number (*e.g.* monophenol, di-phenol, polyphenol, flavonoid or a phenolic acid) or position of side chain attachments of the hydroxyl groups. However, the majority of the compounds proved to be more potent antagonists of ABA in light even at much lower concentrations than in darkness; *e.g.* ferulic acid

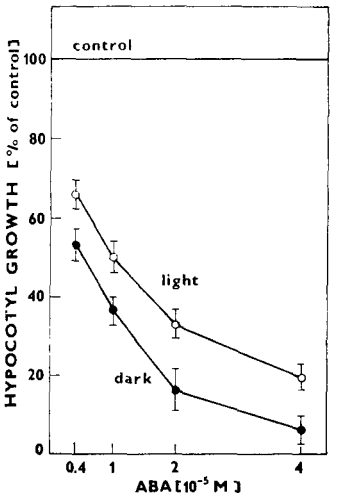

Fig. 1. Inhibition of hypocotyl growth (measured after 72 h) caused by ABA in the light and the dark.

(4 hydroxy- 3 methoxy cinnamic acid) at 10^{-5} M concentration completely counteracted ABA-effect (at 10^{-5} M) in light but remained several times less effective in the dark even when applied with a much lower concentration of ABA (4×10^{-6} M) (Figs. 2 and 3) and so also the rest of the compounds in the dark.

GA promotes hypocotyl growth in light and the promotion shows a concentration response but remains almost ineffective and causes insignificant promotion in the dark (Fig. 4a). Similarly, the effect of a representative phenolic compound (tannic acid) on hypocotyl extension has been shown in Fig. 4b.

Modulation by CA (cinnamic acid) of GA-ABA interaction has been analysed by keeping GA and CA concentrations fixed at 10^{-4} M and 10^{-5} M, respectively and varying ABA from 4×10^{-6} to 4×10^{-5} M (Fig. 5). Interaction of ABA has also been observed by keeping the GA concentration fixed at 10^{-4} M and varying ABA from 4×10^{-6} to 4×10^{-5} M (Fig. 5). Treatment with 10^{-4} M GA is able to overcome fully the inhibition of hypocotyl growth caused by 4×10^{-6} and 10^{-5} M ABA in light and not in the dark. The reversal of inhibition with GA is reduced in magnitude at higher concentrations of ABA and 10^{-4} M GA is less effective with 2×10^{-5} and 4×10^{-5} M ABA (Fig. 5). GA remains almost ineffective in combination with ABA in the dark (Fig. 5). When cinnamic acid, a phenolic compound is introduced into this interacting system of GA and ABA, it is observed that the efficiency of GA in promoting hypocotyl growth is restored dramatically in light whereas GA remains

almost inactive in darkness. CA successfully intervenes interacting ABA-GA system to minimize ABA action and allow GA to be fully expressed. This property of cinnamic acid in minimizing the effect of ABA and allowing the expression of GA is a common feature of six other compounds (Fig. 6, a—f). Chlorogenic, cinnamic, ferulic, salicylic, tannic acids and quercetin

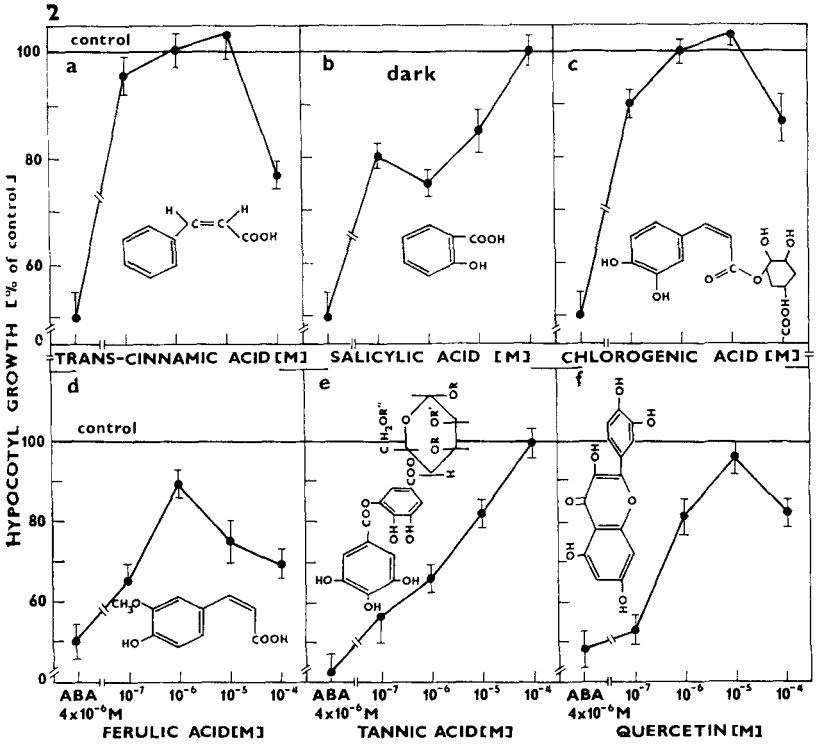

Fig. 2. Recovery of ABA-inhibited hypocotyl growth (after 72 h) by different concentrations of phenolic compounds in the dark. — Abscissae: $4 \cdot 10^{-6}$ M ABA without and with additions of phenolic compounds.

have been applied at low concentrations ranging from 10^{-7} to 10^{-4} M to an interacting system of GA and ABA with fixed concentrations of 3×10^{-5} and 10^{-5} M, respectively (Fig. 6, a—f) in light. The compounds employed nullify the action of ABA on GA-induced growth and favour GA-dominant growth to various degrees. In this concentration range cinnamic and tannic acids were most effective among all the phenolic compounds tested followed by the others. All the compounds discerned highest recovery peak at 10^{-5} M concentration except ferulic acid (10^{-4} M, Fig. 6).

Interaction of ABA and CA has also been observed by keeping CA concentration fixed at 10^{-5} M and varying ABA from 4×10^{-6} to 4×10^{-5} M (Fig. 5) in both light and dark. Treatment with 10^{-5} M CA is able to overcome fully the inhibition caused by 4×10^{-6} and 10^{-5} M ABA in the light (Fig. 5) but at other concentrations, the magnitude of recovery is reduced several times; while in dark-grown seedlings CA antagonizes ABA action completely only at 4×10^{-6} M, the lowest concentration of ABA and the

degree of counteraction slowly decreases as the concentration of ABA increases. The presence or absence of GA along with a phenol does not change the recovery pattern, unlike in light. However, in every case phenolic compounds when present together with GA and ABA antagonize ABA action to various degrees and shift the pattern to GA-like growth.

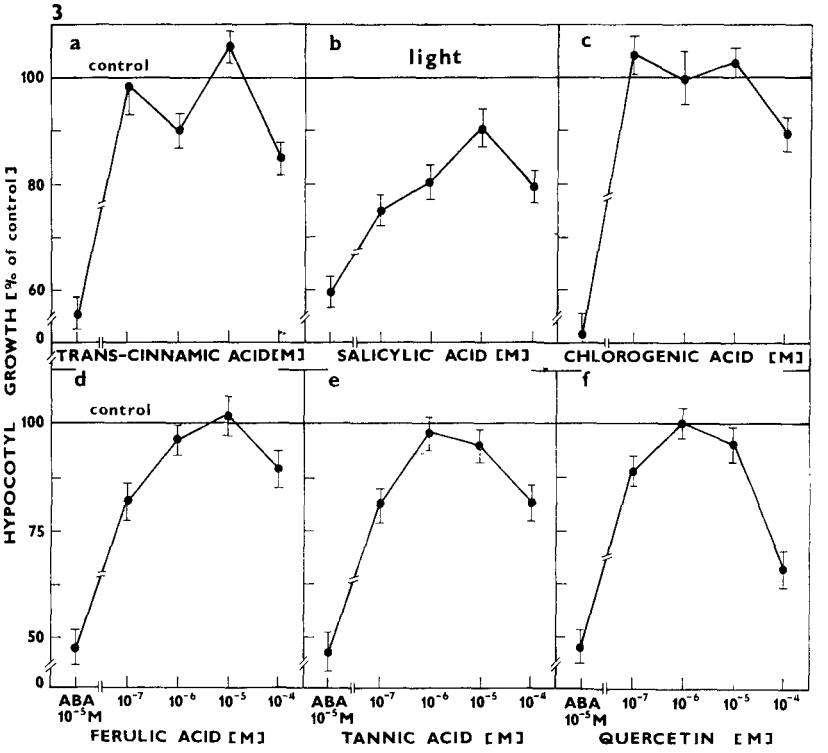

Fig. 3. Recovery of ABA-inhibited hypocotyl growth (after 72 h) by phenolic compounds in light. For captions *cf.* Fig. 2.

The possibility of prevention of ABA uptake by the phenolic compounds has been eliminated by further experiments (RAY 1983). For this, germinated seeds were allowed to grow in 10^{-5} M ABA solution for 48 h either in light or darkness and later transferred to either control or a solution of phenol for the next 36 h. Hypocotyl growth was almost near to the control of the seedlings transferred to phenolic solution; whereas a strong inhibitory action still persisted on the hypocotyl growth in the 48 h ABA pre-treated seedlings on subsequent transfer to controls. However any possible ABA-phenol adduct formation *in vivo* is under close investigation and we have already ruled out such a possibility in our earlier *in vitro* studies (RAY *et al.* 1980).

DISCUSSION

Growth and form of the plants is controlled by an interaction of environmental factors like light, temperature, and the endogenous hormonal activities of certain groups of substances present/produced in different parts of the

plant as determined by the genetic constitution of the individual plants. Growth is the ultimate expression of an interaction between inhibitors and promoters. Our results indicate that a dual role can be assigned to phenolic compounds as inhibitors of growth at higher concentrations and relievers of ABA-suppressed growth at lower concentrations by virtue of their anta-

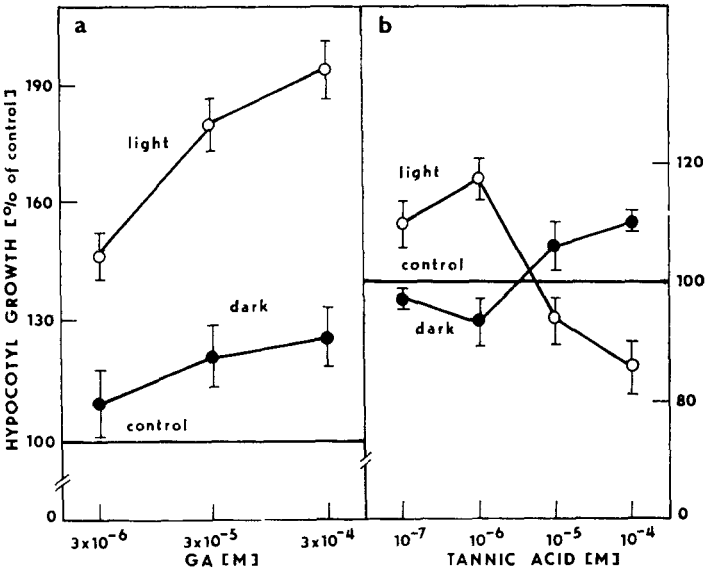

Fig. 4. Effect of GA and tannic acid on hypocotyl growth (after 72 h) in the light and the dark.

gonistic action against ABA. Their promotive action may be due to an indirect result obtained by the release of ABA-imposed inhibition. In all the other studies where phenolic compounds have been shown to inhibit growth, much higher concentrations have been used (CORCORAN 1976, WOLF *et al.* 1976, CHALLICE 1977, MEGHA and LALORAYA 1978, EINHELLIG *et al.* 1982, STOM 1982) which have limited physiological significance. In the present study the effectiveness of phenolic compounds as antagonists of ABA is evident at very low concentration range. Ineffectiveness of GA to reverse ABA inhibition and its subsequent recovery by phenolic compounds indicate the possibility of a true interaction between the two. In counteracting the ABA effect, phenolic compounds mimic the interaction of other growth promoting hormones, *e.g.* IAA and Kn with ABA. KAUFMAN and JONES (1974) have already indicated that GA-ABA interaction is of a non-competitive nature and ABA may be acting at another physiological site from that of gibberellin, as has been supported by numerous other workers. Line Weaver-Burk analysis shows a similar pattern of interaction between GA and ABA even in our studies (data not shown). Therefore, it seems logical to suggest from the present studies that phenolic compounds may be presumably competing with ABA to suppress its action *in vivo* and spare gibberellin to express its action fully. Combined action of GA and ABA has been studied in a variety of physiological processes (MILBORROW 1974, WALTON 1980) and the site of action of ABA has been sought at the level of synthesis

of nucleic acids (Ho and VARNER 1976, WALTON 1980) and DURLEY *et al.* (1976) have already shown the interference of abscisic acid in GA metabolism and uptake, whereas the majority of the reports interpret phenol-induced growth in terms of IAA metabolism (TOMASZEWSKI and THIMANN 1966, and LEE *et al.* 1982: Cf. MOHANKRISHNA and BHARTI 1983) or GA action (CORCORAN 1976).

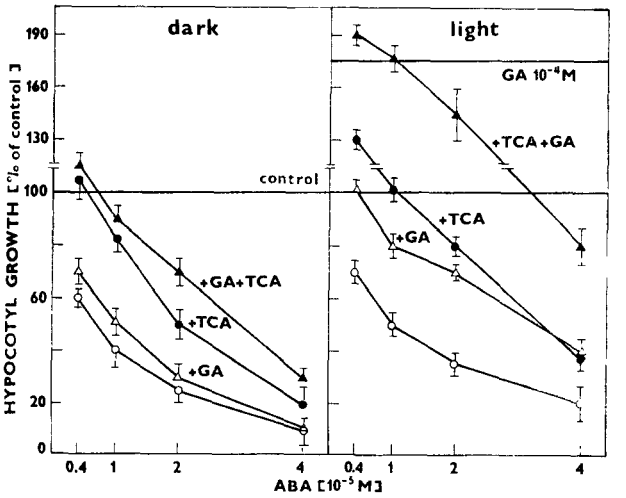

Fig. 5. Interaction of ABA and GA and ABA and TCA in the control of hypocotyl growth (after 72 h) and modulation by TCA of ABA-inhibited GA-induced hypocotyl growth in light and dark.

Synthesis of phenolic/flavonoid compounds is activated in light; whenever any exogenously applied compound is taken up by a plant, it may reach its site of action unaltered or as one or more of its conversion products. It is difficult to emphasize which compound is competing with ABA *per se* to reverse its effect and how. The precise effect of added phenolics on ABA-inhibited growth in light therefore seems difficult to assess. Since biosynthesis of phenolic substances is very limited in dark-grown seedlings; any effect on growth of the externally applied phenolics could be interpreted with some confidence, as it avoids altogether any interference caused by light triggered reactions thus avoiding multiple competitions. So the present study was carried out in both light and dark. Above results clearly demonstrate that the phenolic compounds are more potent in antagonizing ABA effect in light than in darkness and probably phenols synthesized in light synergise exogenously applied phenols to nullify ABA action. Ferulic acid is an interesting example. Another interesting observation is that the action of ABA is more potent in the dark than in light. It may be due to rapid isomerization of ABA in light (WALTON 1980). Further, to confirm the ubiquity of the phenomenon, a flavonoid rich radish has been chosen in the present study (anthocyanin containing) and no consideration has been given to the endogenous levels of phenols/flavonoids. In all our earlier work we have used *Amaranthus*, a betacyanin containing centrospermeae. Therefore, a conclusive assessment of the action of phenolic compounds cannot be made at this moment. It requires further study at molecular level.

It may be suggested from the present studies that the growth retardation, scenescence, dormancy and abscission-inducing potency of abscisic acid and its counteraction by phenolic compounds may reflect changes observed in the endogenous quantities of ABA during seasonal variations. Phenolic compounds may operate by modifying the action of ABA (RAY *et al.* 1980,

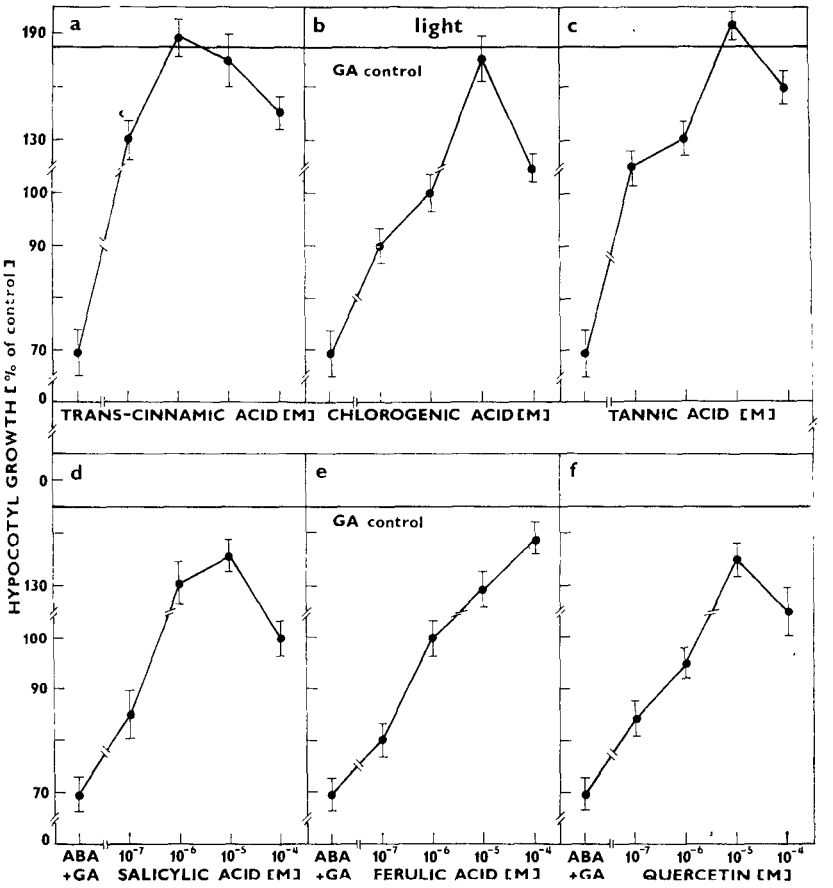

Fig. 6. Modulation of GA-ABA interaction (ABA 10^{-5} M + GA 3×10^{-5} M) by phenolic compounds in light.

APTE and LALORAYA 1982, RAY 1983, PAWAR *et al.* 1984), cytokinins (RAY *et al.* 1983) and gibberellins (CORCORAN 1976 and the present studies) during plant growth and several physiological effects could be explained with this twin action of phenols observed in the plant dvelopment. Phenolic compounds appear to realize their biological potency in balancing the effect of inhibitors/promoters during plant growth.

Acknowledgements

Financial assistance by CSIR, New Delhi (a post-doc to SDR) during a part of this work, and other helps extented by Professor S. Ji are gratefully acknowledged.

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