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Short communication

Enhancement of adventitious root formation in mung bean cuttings by 3,5-dihalo-4-hydroxybenzoic acids and 2,4-dinitrophenol

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Abstract. 3,5-Dihalo-4-hydroxybenzoic acids enhanced adventitious root formation in mung bean (*Vigna radiata* L.) cuttings. 3,5-Diiodo-4-hydroxybenzoic acid was more active than 3,5-dichloro-4-hydroxybenzoic acid, increasing the number of roots formed by about 4-fold. 2,4-Dinitrophenol also enhanced significantly adventitious root formation in mung bean cuttings. The phenolic compounds were active with or without indole-3-acetic acid. The possible mechanism by which these phenolic compounds enhance rooting is discussed.

Abbreviations: CCCP, carbonyl cyanide 3-chlorophenylhydrazone; DIHB, 3,5-diiodo-4-hydroxybenzoic acid; DNP, 2,4-dinitrophenol.

1. Introduction

The stimulation of adventitious roots by phenolic compounds is well documented [5, 7]. Many of these compounds act synergistically with added auxin and therefore the term auxin-synergists has been used to describe these chemicals. However, some phenolic compounds enhance rooting when applied alone [3, 8, 10]. Based on studies with mung bean cuttings, it has been suggested that the structural requirement for a phenolic compound to stimulate rooting is an ortho positioning of hydroxyl groups and a free para position [6]. Although many reports confirm the activity of ortho-diphenols, some observations question the significance of this structural requirement [2, 3, 4, 9].

We report here that 3,5-dihalo-4-hydroxybenzoic acids and 2,4-dinitrophenol, which are not derivatives of ortho-diphenols, are active in promoting adventitious root formation in mung bean cuttings.

2. Materials and methods

2.1 Plant material

Mung bean (*Vigna radiata* L.) seeds were soaked in tap water for 18 h and then sown in moistened Vermiculite. Seedlings were grown at 25 °C and 70% relative humidity and a 16-h photoperiod with a quantum flux density of 170 $\mu E\, m^{-2} sec^{-1}$ (cool white fluorescent tubes).

2.2 Rooting system

Cuttings were made from 9-day-old seedlings. A cutting consisted of a terminal bud, two primary leaves, epicotyl, and 4 cm of the hypocotyl. Four cuttings were placed in a 25-ml vial containing 15 ml of distilled water or test solutions. The cuttings were treated with phenolic compounds with or without 30 μM IAA for 24 h and were then transferred to distilled water. The distilled water was renewed every 24 h. Rooting was performed at 25 °C and 75% relative humidity and a 16-h photoperiod with a quantum flux density of 380 $\mu E\, m^{-2} sec^{-1}$ (cool white fluorescent tubes and incandescent bulbs). Number of visible roots was determined 7 days after the cuttings were made. Results are reported as means of 6 vials.

3. Results and discussion

Under the rooting conditions utilized in this study, IAA alone, applied at 30 μM, had a slight inhibitory effect on adventitious root formation (Tables 1 and 2). 4-Hydroxybenzoic acid somewhat stimulated rooting at high concentrations (Table 1). 3,5-Dichloro-4-hydroxybenzoic acid showed an intermediate activity in inducing rooting, whereas 3,5-diiodo-4-hydroxybenzoic acid (DIHB) was the most active. DIHB also accelerated the emergence of adventitious roots (data not shown). Both substituted phenolics stimulated rooting with or without IAA. The dichloro derivative showed even higher activity without IAA than in the presence of IAA. The reason for the reduction in adventitious root formation in seedlings receiving the combined treatment is yet unclear. In contrast to the high rooting activity of DIHB, 3,5-diiodosalicylic acid was almost inactive. Salicylic acid had a slight stimulating effect, as previously reported [10]. The ability of 3,5-dihalophenolic acids to stimulate rooting of mung bean cuttings (Table 1), is similar to the biological activity of these compounds in other plant systems. DIHB was found to be more active than 3,5-dichloro-4-hydroxybenzoic acid

Table 1. Effect of Phenolic acids on adventitious root formation in mung bean cuttings.

Phenolic acid	Conc. µM	Number of roots/cutting	
		H_2O	IAA
4-Hydroxybenzoic acid	0	15.0 ± 1.4[a]	13.7 ± 0.9
	100	14.6 ± 0.8	14.0 ± 1.3
	200	17.8 ± 1.0	16.2 ± 1.4
	500	21.8 ± 2.1	23.5 ± 1.4
3,5-Diiodo-4-hydroxybenzoic acid	0	14.2 ± 1.4	13.0 ± 1.0
	50	36.1 ± 1.7	30.8 ± 2.3
	100	50.4 ± 2.8	44.5 ± 1.9
3,5-Dichloro-4-hydroxybenzoic acid	0	13.2 ± 0.6	11.5 ± 0.9
	50	19.9 ± 1.0	16.3 ± 0.7
	100	30.1 ± 1.4	16.6 ± 1.2
	200	36.6 ± 2.3	14.2 ± 1.0
Salicylic acid	0	14.7 ± 0.6	12.5 ± 1.0
	100	18.4 ± 1.1	19.2 ± 1.5
	200	23.5 ± 0.9	21.1 ± 1.4
3,5-Diiodosalicylic acid	0	13.9 ± 0.8	11.8 ± 0.9
	50	17.9 ± 1.3	12.4 ± 0.7
	100	7.9 ± 0.9	11.9 ± 1.2

[a] Mean ± SE (n = 6).

in promoting the elongation of cress roots under light, whereas 3,5-diiodosalicylic acid was almost inactive [16, 18]. DIHB has been extensively studied in relation to root growth and gravireaction, but its mode of action has not been clearly elucidated [1].

The above data demonstrating the rooting ability of dihalo-4-hydroxybenzoic acids (Table 1) are inconsistent with the structural requirement defined by Hess [6], i.e. a phenolic compound possessing adjacent hydroxyl groups and a free para position on the aromatic ring. Both the dichloro and diiodo derivatives are monophenols and do not possess a free para position. Salicylic acid and its derivatives are more closely related to these structural requirements, possessing a hydroxyl group adjacent to a carboxyl group, which is also a weak acid, and a free para position. However, these chemicals are much less active and even inhibit rooting at high concentrations. Other reports also questioned the significance of this structural requirement [2, 3, 4, 9].

The observations that dihalo-4-hydroxybenzoic acids were active without exogenous IAA do not negate the term auxin-synergists which has been used to describe phenolic compounds with rooting capability. It is possible that these compounds act synergistically with endogenous IAA [8].

Table 2. Effect of uncouplers on adventitious root formation in mung bean cuttings.

Uncoupler	Conc. μM	Number of roots/cutting	
		H_2O	IAA
DNP	0	14.5 ± 0.9[a]	14.1 ± 1.6
	10	39.1 ± 2.1	31.2 ± 1.7
	30	53.9 ± 1.9	55.4 ± 3.1
CCCP	0	14.7 ± 0.7	13.9 ± 0.8
	1	14.5 ± 1.0	14.8 ± 0.8
	3[b]	15.0 ± 1.1	13.7 ± 0.6

[a] Mean ± SE (n = 6).
[b] Higher concentrations were phytotoxic.

The mechanism by which phenolic compounds stimulate rooting is unclear. It may be that phenolic compounds sustain a higher level of free IAA in the tissues by inhibiting conjugation and oxidative degradation of IAA [11], thus stimulating rooting. DIHB was found to inhibit conjugation (J Riov and SF Yang, unpublished) as well as IAA decarboxylation in vivo [17]. There are several reports demonstrating the ability of phenolic compounds to inhibit IAA conjugation [11, 12]. Since various phenolic compounds are capable of uncoupling oxidative phosphorylation [14, 15], IAA conjugation may be hampered by the consequent reduction in the supply of ATP. 2,4-Dinitrophenol (DNP) was reported to inhibit IAA conjugation and this effect was attributed to the uncoupling activity of DNP [13]. Accordingly, we examined the effect of two uncouplers, DNP and carbonyl cyanide 3-chlorophenylhydrazone (CCCP), on rooting of mung bean cuttings. DNP significantly stimulated rooting with or without IAA, whereas CCCP did not show any root promoting activity (Table 2). Because of its high toxicity, CCCP could be used only at relatively low concentrations. Therefore, it is difficult to draw a clear cut conclusion on the possible involvement of uncoupling in the rooting promoting activity of phenolic compounds. Additional data suggest that the stimulation of rooting by DIHB and probably other phenolics is more complex. Firstly, DIHB was found to be a much less effective uncoupler than DNP [15]. Secondly, we used IAA at 30 μM, a concentration which did not affect rooting (Tables 1 and 2). It was expected that if the phenolic compounds act predominantly by inhibiting IAA conjugation, a combined treatment will result in a synergistic effect on rooting due to a higher level of free IAA in the treated seedlings. However, the data did not confirm this hypothesis. Thirdly, DIHB sustained a higher level of free IAA in the cuttings only during the first few h of the rooting process (J Riov and SF Yang, unpublished).

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