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Short Communication

An Examination of the Growth Substance Activity of Vitamin D_3[1]

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Abstract. The plant growth activity of vitamin D_3 has been examined using well-established bioassays. Vitamin D_3 was found to promote adventitious root formation in cuttings of *Populus tremula* and to promote the germination of lettuce (*Lactuca sativa* cv. Grand Rapids) seeds in the absence of light. No other effects were observed typical of plant growth substances.

Vitamin D_3 (Vit D_3) and vitamin D_3-like substances have been shown to enhance adventitious rooting in *Populus tremula* (Buchala and Schmid 1979, Pythoud et al. 1986), *Phaseolus aureus* (Jarvis and Booth 1981), and *Cynara scolymus* (Moncousin and Gaspar 1983). On simultaneous application to cuttings of *P. tremula*, Vit D_3 acts synergistically with indolylbutyric acid (IBA) (Pythoud et al. 1986). Although Vit D_3 or hydroxylated derivatives of Vit D_3, in the form of glycosides, have been reported in various plant tissues (Boland 1986), their role is unknown. As a first step to understanding the nature of the plant growth action of Vit D_3 we have compared its effect with those known for other plant growth substances in well-established bioassays and on rooting in *P. tremula*. The effect of common plant growth substances on rooting in *P. tremula* has also been examined.

Materials and Methods

The following bioassays were carried out as described in the citations with

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aqueous solutions of Vit D_3 in the concentration range 0.1–100 mg/l and the corresponding growth substance at appropriate concentrations: (1) cell elongation test for auxins with *Avena sativa* coleoptiles (Smith and Van Staden 1978), (2) α-amylase secretion in embryoless grains of *Hordeum vulgare* for gibberellins (Coombe et al. 1967), (3) dark germination of *Lactuca sativa* seeds for gibberellins or cytokinins (Kahn 1960), (4) senescence in leaves of *Rumex obtusifolius* for gibberellins (Whyte and Luckwill 1966), (5) acceleration of chlorophyll formation in cotyledons of *Cucumis sativa* for cytokinins (Fletcher and McCullagh 1971), (6) cell expansion in cotyledons of *Raphanus sativa* for cytokinins (Letham 1971), and (7) radial swelling and hook maintenance in hypocotyls of *Pisum sativum* for ethylene (Stewart et al. 1974).

Where effects were observed with Vit D_3, the tests were carried with several series of plant tissue, and the average values are given. The effect of far-red light on the promotion of the germination of the lettuce seeds was studied by irradiating the seeds, which had been inbibed in water for 1 h and then treated with Vit D_3 or GA_3 at 5 mg/l for 1 h, with far-red light (Schott & Genossen, Mainz, FRG, RG 9 filter) for 3–8 h. Alternatively, the seeds were irradiated with far-red light for 3 h after the inhibition period and then treated with Vit D_3 or GA_3 in the dark. In all cases, germination was allowed to continue for 3 days in the dark at ~25°C before examination.

The rooting test with herbaceous cuttings of *P. tremula* was as described by Pythoud et al. (1986). Essentially, the plant growth substances were applied basally for 24 h in aqueous solution at the concentrations indicated in the text, which were generally optimum. The cuttings were transferred to distilled water, and the adventitious roots formed were counted after 18–20 days. At least four series of 10 cuttings were used for the values reported.

Results and Discussion

The complex interaction between various plant growth substances and the diverse physiological results obtained with them do not facilitate the identification of the effect of application of a putative new substance. Nevertheless, the first step to understanding any such effect may be obtained by comparison with known hormones in particular bioassays. In this communication these were chosen partly for their specificity but also for their sensitivity, with the aim of covering as many as possible of the reliable tests.

Treatment of *P. tremula* cuttings with abscisic acid (ABA), gibberellic acid (GA_3), ethrel, or 6-benzylaminopurine (BAP) did not promote adventitious rooting (Table 1), and in fact BAP and GA_3 were inhibitory. On the other hand, treatment with indolylacetic acid (IAA) at 40 mg/l, IBA, or naphthalene acetic acid (NAA) at 10 mg/l enhanced rooting, and the results were somewhat similar to those obtained with Vit D_3 at its optimum concentration of 10 mg/l; Vit D_2 did not give results significantly different from those obtained with Vit D_3 (not shown). Vit D_3, however, was not found to induce elongation (Smith and Van Staden 1978) or curving of oat coleoptiles and thus did not appear to act like auxins. That IAA is less efficient than IBA or NAA may be due to its metabolism by peroxidases in *P. tremula*.

Table 1. The effect on adventitious root formation of applying various plant growth substances to cuttings of *Populus tremula* with and without vitamin D_3 at 10 mg/l.

Plant growth substance	Concentration (mg/l)	Number of roots/cutting	
		− Vit D_3	+ Vit D_3
Vit D_3[a]	10	—	19.1 ± 1.9
IBA	10	18.2 ± 1.7	49.8 ± 4.6[b]
IAA	40	13.3 ± 3.5	16.9 ± 5.0
NAA	10	14.1 ± 4.8	34.0 ± 13.5[b]
GA_3	1	1.0 ± 0	1.0 ± 0
BAP	0.1	4.9 ± 2.9	9.9 ± 4.3[c]
ABA	1	6.1 ± 2.0	10.5 ± 5.4[c]
Ethrel	1	6.3 ± 2.1	18.3 ± 9.1[b]

The control value (no plant growth substance) was 7.7 ± 0.6 root/cutting.
[a] The absolute values obtained with Vit D_3 (and hence with Vit D_3 and plant growth substance) were lower in winter than during the rest of the year. Some of the values (with ABA, ethrel, and BAP) were obtained in winter, and account has been taken of this fact when assessing whether a simultaneous treatment is additive or synergistic.
[b] Synergy.
[c] Additive effect.

Vit D_3 was found to promote the germination of Grand Rapids lettuce seeds (Table 2) in the absence of light, similarly to GA_3 (Kahn 1960). When imbibed lettuce seeds are treated with far-red light prior to the GA_3, the seeds remain dormant (Negbi et al. 1968). With GA_3 at 5 mg/l, applied after irradiation with red light at 700–1000 nm for 3 h, germination was 15 ± 5% compared to the dark control value of 50 ± 12%. With Vit D_3 at 10 mg/l, under the same conditions, germination was 10 ± 5%. If irradiation with the far-red light was applied for a 3–8 h, beginning 1–2 h after the treatment with the growth substances, essentially similar results were obtained. Thus, the effect of far-red light on the promotion of germination of lettuce seeds by Vit D_3 and GA_3 appears similar. However, it should be noted that such far-red reversal is not observed when massive (nonphysiological) amounts of GA_3 are applied (Ikuma and Thimann 1960) and that some non-plant-growth substances—e.g., thiourea, chlorcamphenicol, and organic solvents—also break the dormancy of lettuce seeds (Khan and Tao 1978). In addition, Vit D_3 does not induce the secretion of α-amylases from the aleurone layer of embryoless half barley grains (Coombe et al. 1967), nor does it retard the leaf senescence in *Rumex obtusifolius* L. (Whyte and Luckwill 1966), thereby excluding that Vit D_3 acts like gibberellins.

Cytokinins also promote the germination of the lettuce seeds, but not in the *total* absence of light (Miller 1958). Other tests for cytokininlike activity were carried out. Isolated radish (*Raphanus sativa*) cotyledons expand in response to cytokinins (Letham 1971), and neither auxins nor gibberellins interfere to a significant extent in such expansion. Vit D_3 at 100 mg/l gave rise to an increase in weight of 36% over the control compared to an increase of 50% produced by BAP at 5 mg/l under the same conditions. However, concentrations of 100 mg/l can hardly be considered physiological. Cytokinins also stimulate the syn-

Table 2. Comparison of Vit D_3 and gibberellic acid in the lettuce seed dark germination test.

Concentration (mg/l)	Percent germination[a]	
	Vit D_3	GA_3
20	91 ± 13	92 ± 9
10	84 ± 8	92 ± 8
1	52 ± 12	52 ± 5
0.1	37 ± 13	56 ± 5
0.01	25 ± 9	36 ± 6

The control value (no plant growth substance) was 12% ± 7.
[a] Average values for at least five series of 25 seeds.

thesis of protein and chlorophyll in young tissues (Fletcher and McCullagh 1971). Vit D_3 did not accelerate the greening of cucumber (*Cucumis sativa*) cotyledons, and concentrations greater than 10 mg/l were even found to inhibit chlorophyll formation, whereas BAP at 1 mg/l produced 60% more chlorophyll. A further indication that Vit D_3 does not possess cytokininlike activity was its inability to overcome apical dominance in pea (*Pisum sativum*) seedlings.

In some cases, ethylene (or its synthetic precursor ethrel) has been shown to promote adventitious rooting in herbaceous cuttings (e.g., Robbins et al. 1985). Although a negative response to ethrel was found in *P. tremula*, the effect of the two substances was also compared. However, at concentrations of up to 100 mg/l, Vit D_3 did not produce the typical radial swelling in the apical region of etiolated *Pisum sativum* hypocotyls (Stewart et al. 1974), nor did it give rise to hook maintenance. Clearly, Vit D_3 is not like ethylene in its action.

The effect of simultaneous treatment on cuttings of *P. tremula* with Vit D_3 and various plant growth substances was also examined (Table 1). The synergy between Vit D_3 and synthetic auxins is evident, whereas with IAA the effect was found to be barely additive. Vit D_3 at 10 mg/l also efficiently relieves the inhibition observed with BAP at 0.1 mg/l and ABA at 1 mg/l but without significant rooting promotion, whereas the inhibition due to GA_3 and 1 mg/l was not overcome. Ethrel at 10 mg/l inhibits rooting, whereas at 1 mg/l no effect is observed. However, in the presence of Vit D_3 at 10 mg/l, rooting promotion is observed, and with ethrel at 1 mg/l the effect is significant and synergistic, suggesting some kind of interplay between auxins, ethylene, and Vit D_3.

Conclusion

The results described above indicate that the effect of Vit D_3 cannot be ascribed to that of a particular group of known plant growth substances. Additional bioassays must be carried out to clarify its mode of action. In general, Vit D_3 and some of its derivatives appear to affect several different growth processes, and it is tempting to compare its action to that of brassinosteroids,

which promote growth (elongation and swelling) in the second internode of *Phaseolus vulgaris* (Thompson et al. 1982) and which act synergistically with auxins (Cohen and Meudt 1983) by stimulating auxin-induced ethylene production (Arteca et al. 1983). Glucocorticosteroids have also been shown to stimulate growth (root and shoot elongation) and promote adventitious rooting in intact *Vigna radiata* seedlings (Geuns 1982). These effects have been suggested to arise from increased RNA synthesis; such an increase has been observed in *Vigna radiata* on application of Vit D_2 (Jarvis et al. 1985), and a similar effect is to be expected for Vit D_3.

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