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BRIEF COMMUNICATION

KOH AND H_2SO_4 HAVE NO STIMULATING INFLUENCE ON ADVENTITIOUS ROOT FORMATION IN BEAN PETIOLES

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SUMMARY

Treatment with KOH or H_2SO_4 of the lower part of petioles of leaf cuttings of *Phaseolus vulgaris* before treatment with indoleacetic acid decreased the number of adventitious roots formed on the petioles.

1. INTRODUCTION

Before adventitious roots can be formed mature parenchyma cells must dedifferentiate. Following this dedifferentiation a group of meristematic cells is formed by cell division and induction of dedifferentiation and cell division in neighbour cells, and from this group a primordium arises (MOUREAU 1939, GRAMBERG 1971, OPPENOORTH 1976, 1978).

SOEKARJO (1965) suggested that dedifferentiation can be induced by unspecific agents such as KOH and H_2SO_4; the processes following dedifferentiation, to wit cell division, the organisation of the cells into a primordium, and the outgrowth of the primordium, would be regulated by auxin. After pretreatment with KOH or H_2SO_4 the dedifferentiated cells would be more sensitive to the specific agent for root formation, auxin.

For investigation of the first stage of adventitious root formation an agent that causes dedifferentiation of the mature parenchyma cells without inducing the formation of a root primordium would be very valuable. I therefore investigated whether KOH and H_2SO_4 would act as dedifferentiating agents in petioles of leaf cuttings of *Phaseolus vulgaris*.

2. MATERIALS AND METHODS

Studies were carried out on the primary leaves of *Phaseolus vulgaris* L. cultivar Wagenaar. The conditions during the growth of the plants and during the experiments were as described earlier (OPPENOORTH 1976, 1978). Immediately after the cutting of the leaves the blades were cut to a uniform surface of 15.5 cm^2 to prevent influences of differences in leaf blade area (VAN RAALTE et al. 1975). Before the treatment with indoleacetic acid (IAA) the lower part of the petiole was dipped for 30 minutes in 0.1 %, 0.5 % or 1.0 % (w/v) KOH solution, or in

0.1%, 0.5% or 1.0% (v/v) H_2SO_4 solution, as used by SOEKARJO (1965). The control was treated with demineralized water. To prevent a large uptake of the solution into the transpiration stream the leaf blades were thoroughly sprayed with demineralized water before the pretreatment. In three experiments the petioles were immersed for 16 hours in 10^{-9} M IAA (Merck, Darmstadt, W. Germany) dissolved in a 0.02 M potassium phosphate solution at pH 4.7, in two experiments the petioles were treated with 10^{-4} M IAA for 30 minutes to stimulate root formation.

Eight days after the cutting of the leaves the number of roots formed was counted, the damaged part of the petiole was measured and the distance between the damaged part and the root closest to the leaf blade was measured.

3. RESULTS

Regardless of the number of roots formed by the control, the number of roots produced after pretreatment with KOH or H_2SO_4 was comparable or, as in most cases, less. The density of the roots, i.e. the number of roots per centimetre of petiole with roots, was always lower after pretreatment. The length of the petiole damaged by the pretreatment increased with the increase in concentration. H_2SO_4 was more damaging than KOH.

The results are given in *table 1* for the low IAA concentration and in *table 2* for the high IAA concentration.

4. DISCUSSION

After pretreatment with KOH or H_2SO_4 bean petioles formed less adventitious roots. This contrasts with the findings of SOEKARJO (1965), who reported an increase in the number of adventitious roots formed on the stems of *Coleus scutellarioides* after treatment with KOH or H_2SO_4. In his material the situation is complicated by the presence of quiescent adventitious root primordia in the

Table 1. The results of treatments with KOH and H_2SO_4 before treatment with 10^{-9} M IAA. Number of petioles per treatment (n) with number of rooted petioles between parentheses, total number of roots (R_T), mean length in mm of petioles killed by the pretreatment (L_+), the density of the roots (R/L_R). Three experiments.

Pretreatment	n	R_T	L_+	R/L_R	n	R_T	L_+	R/L_R	n	R_T	L_+	R/L_R
water	10 (10)	217		40.0	10 (3)	22		21.7	10 (7)	57		32.0
0.1% KOH	10 (10)	210	0.1	42.5	10 (1)	1	0	5.0	10 (7)	61	0	25.8
0.5% KOH	10 (10)	101	1.0	18.6	10 (0)	0	1.4		10 (4)	17	5.1	9.4
1.0% KOH	10 (10)	58	2.1	11.3	10 (0)	0	3.9		10 (4)	25	11.5	13.4
0.1% H_2SO_4	10 (10)	149	1.1	18.6	10 (4)	5	1.0	4.3	10 (7)	21	2.1	6.1
0.5% H_2SO_4	10 (10)	162	12.4	16.5	10 (1)	9	8.3	12.9	10 (3)	21	23.6	17.5
1.0% H_2SO_4	10 (9)	103	20.7	19.5	10 (1)	1	11.8	10.0	10 (3)	32	26.4	27.3

Tabel 2. The results of treatments with KOH and H_2SO_4 before treatment with 10^{-4} M IAA are given. Number of petioles per treatment (n) and number of petioles with roots between parentheses, total number of roots formed (R_T), mean length of petiole in mm killed by the pretreatment (L_+), the density of the roots (R/L_R). Two experiments.

Pretreatment	n	R_T	L_+	R/L_R	n	R_T	L_+	R/L_R
water	10 (9)	435		17.3	10 (9)	391		24.4
0.5% KOH	10 (6)	186	1.7	8.6	10 (8)	105	1.7	10.5
1.0% KOH	10 (9)	189	6.5	7.6	10 (9)	201	3.7	14.2
0.5% H_2SO_4	10 (7)	275	8.9	14.2	10 (10)	320	9.5	18.9
1.0% H_2SO_4	10 (2)	5	35.0	7.9	10 (10)	340	15.9	33.4

nodes. The developing nodal root primordia inhibit the formation of adventitious root primordia in the internodes, and Soekarjo suggested that the growing nodal root primordium consumes so much IAA that the concentration necessary for dedifferentiation is not reached in the internode. His suggestion is supported by the results of WARREN WILSON & WARREN WILSON (1977), who reported that removal of emerging adventitious roots stimulates the formation of more adventitious roots on *Sambucus nigra* cuttings.

Bean petioles have no quiescent adventitious root primordia and their anatomy is uniform over the length of the petiole (OPPENOORTH 1976). After pretreatment with KOH or H_2SO_4 bean leaf cuttings formed less roots. The density of the roots was less after these pretreatments. This indicates that KOH and H_2SO_4 not only kill the lower part of the petiole but also damage tissues higher up in the petiole, since the density is a constant within experiments (Oppenoorth, in preparation).

The differences in numbers of roots formed by the controls of the experiments may be caused by environmental differences during the growth of the parent plants. Especially the amount of irradiance influences the rooting of the cuttings, a high irradiance of the parent plant decreases the number of roots formed by the cutting (HANSEN & ERIKSEN 1974, Oppenoorth, in preparation). Regardless of these differences the reaction to treatment with KOH and H_2SO_4 is the same in all experiments: less roots are formed.

A closer analysis of Soekarjo's data shows that the increase in number of roots after pretreatment with KOH or H_2SO_4 is as large as the increase in number of roots after removal of the lowest node or the removal of the lowest node plus a part of the internode. The increase in number of roots formed by *Coleus* stems after treatment with KOH or H_2SO_4 can therefore be explained by the elimination of the quiescent nodal root primordia.

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