Planta 142, 207–210 (1978)

Planta

Effect of Growth Regulators and Role of Roots in Sex Expression in Spinach

M.Kh. Chailakhyan and V.N. Khryanin

K.A. Timiryazev-Institute of Plant Physiology (Academy of Sciences of USSR), Botanicheskaya ul. 35, Moscow I-273, USSR 127273

Abstract. When 7-d-old plantlets of spinach (*Spinacia oleracea* L.) were immersed with their roots for 24 h in 25 mg/l gibberellic acid (GA_3), or 15 mg/l 6-benzylaminopurine (6-BAP), or 15 mg/l indole-3-acetic acid (IAA), or 10 mg/l abscisic acid (ABA) and subsequently grown on long (18-h) days, the ratio of plants with male and female flowers, which in the controls was almost 1:1 (48 and 52%, respectively), was greatly altered. The treatments with 6-BAP, IAA and ABA raised the percentage of female plants to 88, 76 and 71%, respectively; the GA_3 treatment increased the percent of male plants to 79%. When young, vegetative spinach plants (3 visible leaves) grown in 18-h days were cut a the root neck, and the shoots grown with their bases in nutrient solution, with adventitious roots either being allowed to develop or being systematically removed, 85% of the plants without roots became males, 85% of those with roots became females. But if the cut shoots were first, for 28 h, placed in a 15-mg/l 6-BAP solution and then grown in the absence of roots, the percent of female plants was restored to 84. These results fully agree with those obtained previously with hemp, namely, that plant growth regulators exert a regulating effect on the sex expression of dioecious plants when applied through the roots in early stages of development; that the root system plays an important role in determining the sex of these plants, that this role of the roots is associated with the synthesis of cytokinins in them. Dioecious short- and long-day plants do not differ in these respects.

Key words: Abscisic acid – Auxin – Cytokinin – Flowers (sex) – Gibberellin – Sex expression – *Spinacia*.

Introduction, Plant Material

It was shown by us earlier, in experiments with hemp (Chailakhyan and Khryanin, 1978a, b) that growth regulators applied through the roots of plants in an early stage of development caused considerable shifts of the sex ratio, and that the root system of the plants played an important role in determining female sex expression in hemp, this role being evidently connected with cytokinin synthesis in the roots. The question arose whether the results obtained with hemp, a dioecious *short-day* plant, would also be typical of dioecious *long-day* plants.

To resolve this question, experiments with spinach (*Spinacia oleracia* L.) were carried out.

The cultivar used was "Victoria"; the seeds were obtained from Soyuzsortsemovoshch, Ministry of Agriculture of USSR, Moscow.

Methods and Results

a) Effect of Growth Regulators on Sex Expression in Spinach

Two series of experiments were conducted. In the first series, the effect of growth regulators, applied through the roots of the plants, on sex expression in spinach was studied.

For this purpose, seeds were germinated in the dark for 5 d at a temperature of 20° C on water-soaked filter paper. Then, plantlets with rootlets of the same length were placed for 2 d with their roots in 1-l glass containers with water. After this, the plants were transferred for 24 h to solutions of growth regulators while control plants remained in containers with water. The treatments were as follows: (1) control = water; (2) gibberellic acid (GA_3) at 25 mg/l; (3) 6-benzylaminopurine (6-BAP), 15 mg/l; (4) indole-3-acetic acid (IAA), 15 mg/l; (5) $\pm$ = abscisic acid (ABA), 10 mg/l. All regulators were obtained from Sigma Chemical Comp., St. Louis, Mo., USA.

Each treatment included ca. 100 plants. After the 24 h treatment the plantlets were planted in boxes with soil, and were grown on a long (18-h) day until complete visible expression of sex was achieved, under natural light extended with light from xenon-arc lamps (DKsTW 6000, Moscow, USSR; 0.009 W cm^{-2} ~ 14,000 lx) in one of the greenhouses of the Timiriazev Institute.

0032-0935/78/0207/$01.00

Table 1. Effect of growth regulators, applied for 24 h through the roots, on growth and development of spinach plants grown on long days

Treatment	Height of plants ~ (cm)[a]		Appearance of flower buds (d)[b]
	Male	Female	
Control	14.0±1.0	10.0±0.2	14
GA_3, 25 mg/l	25.5±1.7	13.0±1.4	11
6-BAP, 15 mg/l	6.0±0.1	6.0±0.4	20
IAA, 15 mg/l	16.5±1.1	12.0±0.8	14
ABA, 10 mg/l	11.5±0.5	6.5±0.1	17

[a] Mean±standard error
[b] After start of hormone treatment

Table 2. Effect of growth regulators, applied for 24 h through the roots, on sex expression in spinach plants grown on long days

Treatment	% of plants, ±standard error		
	Male	Female	Intersexes
Control	48.3±1.7	51.7±2.3	–
GA_3, 25 mg/l	78.8±3.2	16.3±0.6	4.9±0.3
6-BAP, 15 mg/l	11.2±0.8	86.7±2.9	2.1±0.0
IAA, 15 mg/l	20.8±0.1	76.0±2.2	3.2±0.6
ABA, 10 mg/l	29.0±1.4	71.0±1.8	–

Measurements of plant height showed that GA_3 enhanced mainly the growth of male spinach plants. The growth of both male and female plants was considerably inhibited by 6-BAP and less so by ABA, at the concentrations used, while otherwise the height of the plants was the same as in the controls (Table 1).

The appearance of flower (inflorescence) buds on the plants was retarded under the influence of the 6-BAP and the ABA treatments by 6 and 3 d, respectively, as compared with the controls. The GA_3 treatment, in contrast speeded up the beginning of bud appearance by 3 d (see Table 1). In all cases, both male and female inflorescences appeared after the formation of the 8th visible leaf on the main shoot.

The application of the plant growth substances through the roots of young plants exerted a striking influence on sex expression in the adult plants, the nature of the response depending the particular growth substance (Table 2). Application of GA_3 considerably increased the percent of male individuals (to 79%). Application of 6-BAP, IAA and ABA yielded a majority of female plants (87, 76 and 71%, in this order). In water-treated controls, male and female plants were present in nearly equal numbers (48 and 52%, respectively). Figure 1 illustrates the appearance of control, GA_3- and 6-BAP-treated plants, selected to reflect, approximately, the sex ratios obtained. The figure also shows clearly the growth-reducing effect of the 6-BAP treatment, particularly evident in the male plant on the extreme right, in which the inflorescence can be seen sitting on a greatly shortened stem.

Fig. 1. Influence of growth regulators, absorbed through the roots, on growth and sex expression in spinach plants. Treatment for 24 h, 7 d after the start of germination (seed soaking). Plants grown on 18-h days. Top: control plants treated with water (2 plants on left=female, 2 on right=male). Center: plants treated with GA_3, 25 mg/l (3 plants on left=male, plant on right=female). Bottom: plants treated with 6-BAP (3 plants on left=female, plant on right=male)

The results are thus very similar to those obtained in the experiments with hemp (Chailakhyan and Khryanin, 1978 a, b), except that at the concentrations used the effect of GA_3 on the determination of male plants was relatively stronger in hemp than in spinach and, in contrast, the effect of 6-BAP on determination of female plants was greater in spinach than in hemp.

b) Role of Roots in Sex Expression in Spinach

The second series of experiments was designed to study the role of the root system in the sex expression of spinach plants grown on long days.

Spinach plants were first grown in boxes with soil in the greenhouse on long (18-h) days, as above. When the 3rd leaf became visible, a stage at which the shoot apex of the spinach plants is in a strictly vegetative condition, part of the plants were cut off at the root neck while the remainder were left to grow in the soil. The cut shoots were divided into three groups, and those of two groups were placed with their cut ends into containers with water while those of the third group were kept for 28 h in a 15 mg/l 6-BAP solution. After that, the plants of all three groups were transferred first to 0.1-strength Knop nutrient solution, after 2 d to half-strength Knop, and after 2 more days to full-strength Knop. The nutrient solution was aerated daily. During subsequent growth, secondary (adventitious) roots were allowed to develop on the plants of one of the first two groups whereas they were systematically removed in those of the second of those groups and of the third one.

The containers with the plants were maintained in controlled-environment cabinets under a regime of 16 h light daily (fluorescent lamps; LBZ-60, Baku, USSR; 0.002 W $cm^{-2} \sim 600$ lx). 20° C, and 80% relative humidity. As controls, intact plants left growing in soil were observed so that there were altogether four treatments: (1) intact plants in soil (controls); (2) plants (cut-off shoots) *with* secondary roots; (3) plants *without* roots; (4) plants without roots, treated with 6-BAP.

Table 3. Number of male and female spinach plants with and without roots

Treatment	Male plants		Female plants	
	No.	%	No.	%
Intact plants in soil (controls)	52	47.3	58	52.7
De-rooted plants (in nutrient solution)				
With secondary roots	7	15.0	96	85.0
Without sec. roots	85	85.0	15	15.0
Without sec. roots, +6-BAP	16	16.4	82	83.6

As can be seen in Table 3, plants left to grow in soil on long days (treatment 1) developed into male and female individuals in approximately numbers. Plants in which the root system had been removed but secondary roots had been allowed to develop (treatment 2) became predominantly females whilst plants in which the primary root system *and* the secondary roots had been removed (treatment 3) developed predominantly into males. But if plants the primary root system of which had been removed were treated with 6-BAP, a cytokinin, and thereafter kept in the rootless condition (treatment 4) the sex ratio was the same as in plants with secondary roots. Thus, cytokinin treatment had the same effect on sex expression in spinach as the presence of secondary roots. It may be noted that regeneration of secondary roots in spinach began quite early, 3-6 d after the start of the experiment (i.e. after cutting off the plants from the primary root system). Flower (inflorescence) buds appeared in all cases, i.e. whether or not the secondary roots were removed, 7-10 d after the start of root regeneration. Treatments 2, 3 and 4 are shown in Figure 2.

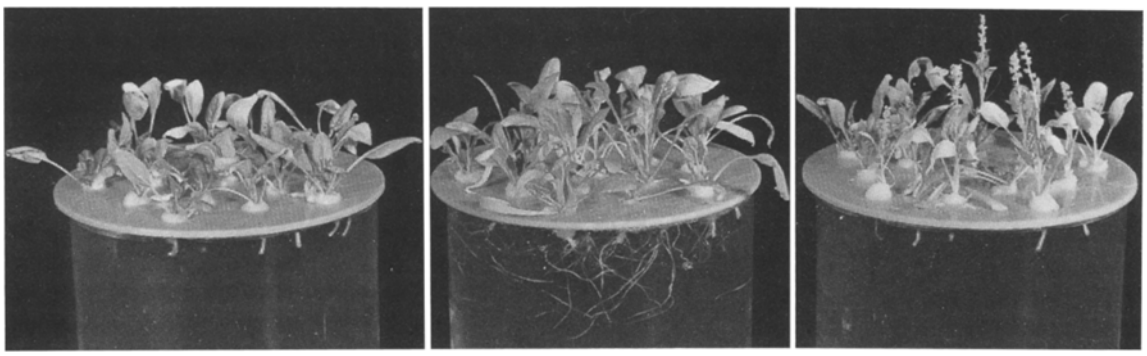

Fig. 2. Influence of roots and of cytokinin treatment on sex expression in spinach plants. Left, plants with adventious roots removed, treated for 28 h with 15 mg/l BAP (treatment 4); middle, plants with adventitious roots allowed to develop (treatment 2); right, plants with adventitious roots removed (treatment 3)

Conclusions

The results of our experiments with spinach agree very closely with our previous results with hemp. They permit us to suggest the following conclusions: *Firstly,* the plant growth substances gibberellin, cytokinin, auxin and abscisic acid exert regulatory effects, characteristic for each of these hormone types, on sex expression in dioecious plants when applied through the root system in early stages of development. *Secondly,* the root system of these plants plays an important part in determining the sex of the plant, and this role is evidently associated with the synthesis of cytokinins in the roots. *Thirdly,* both these effects are quite similar in both dioecious short-day plants and dioecious long-day plants, i.e. are independent of the type of photoperiodic regulation of flower formation.

References

Chailakhyan, M.Kh., Khryanin, V.N.: The influence of growth regulators absorbed by the root on sex expression in hemp plants. Planta **138**, 181–184 (1978a)

Chailakhyan, M.Kh., Khryanin, V.N.: The role of roots in sex expression in hemp plants. Planta **138**, 185–187 (1978b)

Received 10 April; acepted 25 May 1978