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Induction of male sterility in lettuce (Lactuca sativa L.) with GA_3; influence of temperature and GA_3 concentration

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Summary

Induction of male sterility in lettuce by GA_3 application was more successful at varying day and night temperatures than at constant temperatures. In the glasshouse with varying temperatures plants with good habit and practically normal-looking male-sterile flowers were obtained which produced a reasonable amount of seeds after cross pollination. At constant temperatures 20 °C gave the highest frequency of male sterile flowers.

GA_3 concentration influenced both number of flowers per plant, percentage of male-sterile flowers, female fertility and the length of the male-sterile period. The optimal GA_3 concentration at 17 and 20 °C was 50 mg/kg, at 23 °C 100 to 200 mg/kg and at varying day and night temperatures about 200 mg/kg.

Further investigations will be carried out on the influence of light intensity, repeated spraying, and genotype.

Introduction

Large-scale crossing of lettuce is greatly hampered because of the composite nature of the flowers. Emasculation is very time-consuming. From preliminary investigations it appeared that application of gibberellic acid (GA_3) on lettuce plants with flower buds of 1-3 mm long induces male sterility (Eenink & Loupias, 1976; Eenink, 1977). Other growth regulators such as maleic hydrazide, thymine and Ethrel and also $AgNO_3$, had not such an effect (Eenink, unpublished).

In this paper results of research on the influence of GA_3 concentration on the occurrence of male-sterile flowers at different temperatures is described.

Material and methods

In glasshouses of the phytotron at constant temperatures of 17, 20 and 23 °C and natural light conditions and in a glasshouse with day and night temperatures varying between 25 and 15 °C *Lactuca sativa* L. cv. Suzan was grown in the summer of

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1976. Plants with flower buds 1-3 mm long were sprayed once with GA_3 solutions in distilled water of 0 (control), 10, 25, 50, 100, 200 and 400 mg/kg with wetting agent. In the phytotron trial all flowers were recorded from the beginning of flowering and classified into male-fertile (MF) and male-sterile (MS) on the basis of their external appearance. Two types of MS flowers were distinguished:

1) MS1 flowers with shrivelled anthers containing some rather normal looking pollen grains which usually are not liberated;

2) MS2 flowers without anthers or with only rudimentary anthers without pollen grains.

Part of both MF and MS flowers of plants in the phytotron and in the glasshouse was pollinated with pollen from anthocyanin containing *L. sativa* cv. Valore to investigate male and female sterility. With each treatment (temperature × GA_3 concentration) two plants were used.

Results

In Table 1 the influence of temperature and GA_3 concentration on the occurrence of male sterility is shown.

From the overall means for number of flowers per temperature it appears that at 20 and 23 °C much more flowers developed than at 17 °C. At 20 and 23 °C the

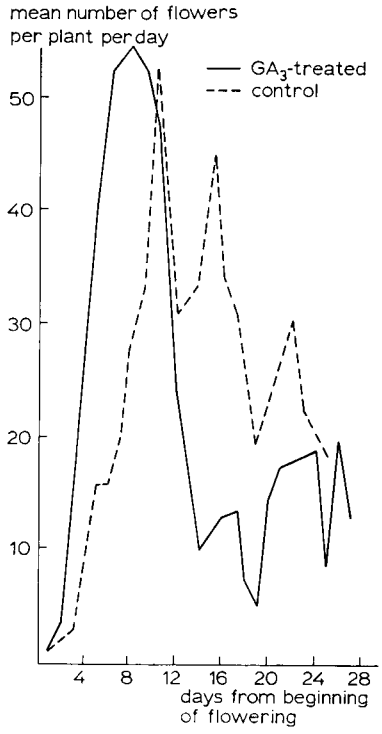

Fig. 1. Mean number of flowers per plant per day of GA_3-treated plants (means of 10-400 mg/kg) and of untreated controls at 20 °C.

Table 1. Percentage of male sterile flowers (MS_1, MS_2, see text) after GA_3 treatment at different temperatures and the total number of flowers per plant (n_1), days from spraying until occurrence of MS flowers (n_2) and the length of the male-sterile period (n_3, in days). Figures are means of two plants per temperature per GA_3 treatment.

GA_3 concentration	17 °C					20 °C					23 °C					Mean				
	MS_1	MS_2	n_1	n_2	n_3	MS_1	MS_2	n_1	n_2	n_3	MS_1	MS_2	n_1	n_2	n_3	MS_1	MS_2	n_1	n_2	n_3
0	0	0	438	—	—	0	0	531	—	—	0	0	381	—	—	0	0	450	—	—
10	1	0	239	19	3	6	1	726	10	6	3	0	599	13	2	3	1	555	14	4
25	9	3	190	13	13	21	6	748	10	14	19	4	476	10	12	16	4	471	11	13
50	16	11	178	16	14	20	21	687	10	18	29	4	637	10	10	22	12	501	12	14
100	15	19	189	16	15	24	53	397	10	19	32	17	369	10	13	24	30	318	12	16
200	10	19	262	15	19	18	54	268	10	21	26	37	256	10	13	18	37	262	12	13
400	2	1	160	15	2	23	62	425	10	13	25	39	274	10	14	20	34	286	12	10
Mean	9	9	237	16	11	19	33	540	10	15	22	17	427	11	11					

Table 2. Mean seed production on male-fertile (MF) and male-sterile (MS_1 and MS_2, see text) flowers from cross pollination or selfing (MF flowers in the glasshouse) after GA_3 application at different temperatures. Figures are means of at least 10 flowers per treatment.

GA_3 concentration	17 °C			20 °C			23 °C			Glashouse			Mean		
	MF	MS_1	MS_2	MF	MS_1	MS_2	MF	MS_1	MS_2	MF	MS_1	MS_2	MF	MS_1	MS_2
0	8.0			7.4			7.3						7.6		
10	3.3	4.0		8.8	5.0		8.0					8.0	5.2	4.5	8.0
25	2.2	1.6	3.0	2.9	3.2	4.0	8.0	2.0	1.0	14.7	13.0	6.0	6.5	4.9	3.5
50	5.5	2.8	3.8	4.0	3.2	4.0	6.6	1.4	2.7		7.5		5.4	3.7	2.8
100	0	0.7	0.5	4.0	0.9	0.5	13.5	3.6	1.6	8.0		6.0	6.4	1.7	2.2
200	4.6	1.0	0.1	0	0	0.1	4.6	3.9	1.0	5.0	8.8	7.1	3.6	3.4	2.1
400	0	0	0	0	0	0	0	1.2	0.2	—	6.8	6.1	0	2.0	1.6
Mean	2.6	1.7	1.5	3.3	2.1	1.7	6.9	2.4	1.3	9.2	9.0	6.3			

number of flowers per GA_3 concentration increased after application of low GA_3 concentrations. Application of high GA_3 concentrations resulted in a decrease of number of flowers.

Fig. 1 shows the distribution of mean number of flowers of GA_3-treated and untreated plants per day at 20 °C and is representative of results at other temperatures. At the beginning of flowering, plants treated with GA_3 flower more abundantly than untreated plants whereas after about two weeks the opposite occurs.

The overall means for percentage of MS flowers – 100 MS/(MS + MF) – per temperature in Table 1 show that at 17 °C significantly fewer MS flowers were produced than at 20 or 23 °C; the highest frequency was obtained at 20 °C. After spraying with 10 and to a lesser extent with 25 mg/kg GA_3 solutions MS flowers were obtained in a low frequency. Application of 100, 200 or 400 mg/kg solutions, especially at 20 °C, resulted in a high frequency of MS flowers.

Time from spraying until the occurrence of the first MS flowers was significantly longer at 17 °C than at 20 or 23 °C but was not clearly affected by GA_3 concentration. Only after application of 10 mg/kg GA_3 solutions at 17 and 23 °C it was longer than at higher concentrations. The period during which MS flowers occurred was significantly longer at 20 °C than at 17 or 23 °C especially after GA_3 application of 100 and 200 mg/kg.

Fig. 2 gives the frequency of MS flowers at 20 °C at different times from the beginning of flowering. It is representative of results at other temperatures and shows that in certain periods all flowers on a plant were sterile.

In Table 2 the mean numbers of seeds per MS1, MS2 and MF flowers for each temperature and GA_3 concentration are given. From the overall means per temperature it appears that in the glasshouse at different day and night temperatures significantly more seeds were formed than at constant temperatures of 17, 20 or 23 °C.

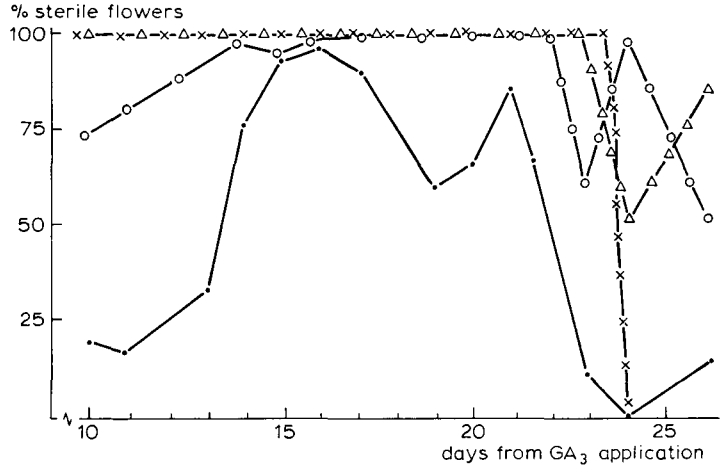

Fig. 2. Mean percentages of sterile flowers per plant per day after application of different GA_3 solutions at 20 °C. GA_3 concentrations:
●——● 50 mg/kg; o——o 100 mg/kg; △——△ 200 mg/kg; ×——× 400 mg/kg.

Seed production after GA_3 treatment both for MF and MS flowers was rather often lowest at 17 °C. Because MF flowers from untreated plants produced about the same number of seeds at 17, 20 and 23 °C, the above suggests that at lower temperatures the negative influence of GA_3 treatment on seed production is greater.

MF flowers produced more seeds than MS flowers. There was no significant difference in seed production of MS1 and MS2 flowers. Only in certain cases MS1 flowers produced more seeds than MS2 flowers, maybe because in MS1 flowers, which show a less disturbed male sex apparatus, also female fertility is less disturbed. In the glasshouse seed production on MS1 and MF flowers was equal.

The overall means for seed production per GA_3 concentration show that, both with MF and MS flowers, seed production was generally lower if higher GA_3 concentrations had been applied.

Cross pollination of MF flowers resulted in most cases in a certain percentage of hybrid seeds, varying between 15 and 50 % independent of GA_3 concentration and temperature. After pollination of MS1 and MS2 flowers usually 100 % hybrid seeds were obtained. Only in a few cases did a small percentage of self seeds occur on MS1 flowers, mostly after application of GA_3 concentrations of 10 to 50 mg/kg. There was no relationship between the occurrence of self seeds on MS flowers and temperature.

Discussion and conclusions

Flower heads developed more slowly at 17 °C than at the higher temperatures and often opened only slightly so that it was difficult to see whether they were male sterile or fertile. At 23 °C plants suffered from GA_3 treatment as was manifested by small flowers and thin flower stalks. Hence, if experiments are carried out at constant temperatures, 20 °C should be preferred in combination with a GA_3 concentration of about 50 mg/kg because of a better plant habit, a better flower quality and a greater number of flowers per plant, a higher frequency of sterile flowers and a longer duration of male sterility. However, induction of male sterility at varying temperatures in a glasshouse with e.g. 200 mg/kg GA_3 solution results in practically normal-looking plants with good seed production on MS flowers and should therefore be preferred.

From other preliminary investigations the impression was gained that besides temperature also light intensity and genotype strongly influence induction of male sterility by GA_3 spraying. For instance low light intensity had a deleterious effect on certain treated plants.

No temperature × GA_3 concentration treatment resulted in completely male-sterile plants, nor were such plants obtained in other experiments with repeated GA_3 spraying. Further investigations on the influence of light intensity, repeated spraying and genotype will be carried out.

Acknowledgment

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