1998

Gibberellic acid suppresses production of vegetative topsets and promotes development of flowers *in vitro* in garlic (*Allium sativum*) inflorescences.

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INTRODUCTION

Garlic (*Alium sativum*) is a sterile plant and it is therefore propagated asexually by means of underground cloves and vegetative topsets in the inlorescences. One possible cause of the sterility could be a competition between flower buds and rapidly growing vegetative topsets in the inlorescence (Koul and Gohil 1970). Alternativelly, Konvicka proposed a hypothesis that male sterility in garlic could be due to a tapetum disease caused by microorganisms (Konvička 1973, Konvička *et al.* 1978). Our previous studies were thus concentrated on testing the hypothesis of chronic microbial contamination of garlic plants (Fellner 1995, Fellner *et al.* 1996). Plant hormones including gibberellic acid are also known to affect the development of reproductive organs (Pharis and King 1985). In this study we report on the effect of exogenous GA_3 on development of inflorescences and flower organs in garlic *in vitro*.

MATERIAL AND METHODS

Cultivated garlic (*Allium sativum* L. cv. "Moravan") grown in natural field conditions in the Czechia was used as a source of inflorescences. Young inflorescence umbels cut-off from curled flowering stalks were surface sterilized for 20 min in 5% Chloramine B (Natrium benzensulfochloramidicum, 1.5% active chlorine) and rinsed five times in sterile distilled water. Sterilized umbels with the rest of stalk were stuck into agar culture medium in Erlenmayer flasks. The basal medium contained macroelements, microelements and vitamins based on BDS medium (Dunstan and Short 1977), and was supplemented with 3% sucrose, 0.1% casein hydrolysate, 10mM MES and 2.5µM kinetin. pH was adjusted by 1M KOH to 5.7 before autoclaving. To this basal medium, gibberellic acid GA_3, antiauxin PCIB and antibiotic ciprofloxacin were added in appropriate concentrations (see results). The inflorescences (2-3 per Erlenmayer flask) were then cultured for 40 days under 16 hr-light period at 21°C, with one subculture on fresh medium after first 20 days of culture. In each experiment 8 to 10 inflorescence umbels of similar initial diameter (5-6 mm) were cultured on each tested effector.

RESULTS AND DISCUSSION

In two independent experiments, effect of gibberellic acid GA_3 (10^{-4}, 10^{-3} M), antiauxin PCIB (2-[p-chlorophenoxy]-isobutyric acid) (10^{-4} M) and antibiotic ciprofloxacin (20 mg/l) on *in vitro* development of young inflorescences was studied. These effectors were applied separately and in one case, 10^{-4} GA_3 was mixed with 20 mg/l of ciprofloxacin. After 40 days of culture the inflorescence umbels were evaluated for several parameters (see Table 1).
In the basal medium the inflorescences increased their diameter about two times and changed their color from starting dark-green to light-brown (Table 1). Inflorescences produced white vegetative topsets of round-heart shape together with low number of white-brown flowers. Anthers were of yellow-brown color and contained non-viable degenerated tetrads.

Application of GA_3 led to change in color of cultured inflorescences, the suppression of development of vegetative topsets in the inflorescences, and an increase in the number of flowers. Flowers developed white or white-green anthers containing young uninucleate microspores with viability between 20 to 30 %. Effect of gibberellic acid was dependent on its concentration. GA_3 in concentration 10^{-3} M totally suppressed development of vegetative topsets and higly increased the number of flowers. At 10^{-4} M GA_3 very narrow pin-shaped vegetative topsets in the inflorescence and still high number of flowers were formed in comparison with the control (Table 1). Similar results were obtained when 10^{-4} M GA_3 was applied simultaneously with ciprofloxacin. The differences from the effect of GA_3 alone was that in the presence of ciprofloxacin the effect of GA_3 on the increase of flower number and decrease of topset diameter was eliminated. However, simultaneously ciprofloxacin alone had only very weak effect on development of garlic inflorescences. Application of antiauxin PCIB did not lead to any significant differences in inflorescence morphology compared with the control.

Together, the results show that GA_3 is involved in the development of garlic flowers *in vitro*, including the normal development of male reproductive organs. In addition, GA_3 controls competition between development of flowers and topsets in the inflorescences *in vitro*. These results are similar to the observation of Tizio (1979) who found that gibberellic acid in association with adenine and biotine stimulates normal development of garlic flowers cultured *in vitro*. The lack of the effect of antiauxin PCIB on garlic inflorescence morphology may suggest that auxins are not probably directly involved in development of this part of garlic plant. Compensation of GA_3 effect by ciprofloxacin on induction of flowers is hardly to explain, especially because not all the inflorescence responses to GA_3 were affected. It is for example possible that ciprofloxacin somehow affects metabolic pathway of applied GA_3 specific for flower induction.

In conclusion, these results show that exogenous GA_3 can promote the development of viable microspores *in vitro*, the process which is affected *in vivo* in the most of cultivated garlic clones.

Acknowledgment

I thank to Professor Vipen K. Sawhney from Department of Biology, University of Saskatchewan in Canada for his comments on the manuscript and for a correction of English language.

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Table 1 : Effect of GA_3, antiauxin PCIB and antibiotic ciprofloxacin on development of garlic inflorescences *in vitro*.

Effector applied to basal medium	Umbel diameter (mm)	Umbel color	Number of topsets	Topset diameter (mm)	Topset color	Topset shape	Number of flowers per inflorescence	Flower color	Anther color	Microspore stage*	Microspore viability*
Control	11.8 ± 0.5	light-brown	27.5 ± 4.7	2.8 ± 0.3	white	round-heart	19.5 ± 5.7	white-brown	yellow-brown	tetrads	0
100 µM GA_3	10.3 ± 0.3	light-green	26.8 ± 8.9	0.9 ± 0.1	white	pin	49.2 ± 3.3	white	white	uninucleate	23.1 ± 1.8
1 mM GA_3	9.3 ± 0.9	light-green	0	-	-	-	93.7 ± 5.0	white	white	uninucleate	27.7 ± 1.5
100 µM PCIB	11.7 ± 0.4	light-brown	30.2 ± 3.0	2.4 ± 0.2	white	round-heart	10.3 ± 5.4	white-brown	white-brown	tetrads	0
20 mg/l ciprofloxacin	11.7 ± 0.7	light-brown	27.3 ± 3.8	2.2 ± 0.1	white	round-heart	22.3 ± 4.8	white-brown	white-brown	tetrads	0
100 µM GA_3 + 20 mg/l ciprofloxacin	8.8 ± 0.5	light-green	28.5 ± 9.6	1.5 ± 0.2	white	pin	25.5 ± 6.1	white-green	white	uninucleate	23.5 ± 2.1

* Microspore viability was tested by FDA (fluorescein diacetate) staining (Widholm 1972), and staining of microspores for determination of developmental stage was made by means of acetocarmine squash preparation.

Allium Improvement Newsletter

1998 / Volume 8

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