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The effects of hormones and saccharides on growth and flowering of green and herbicides-treated *Chenopodium rubrum* L. plants

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Abstract

The medium for *in vitro* culture of green and SANDOZ herbicides-treated *Chenopodium rubrum* L. plants contained saccharides and hormones in different concentrations. Five days after sowing, the plants were exposed to non-inductive (15 long days - LD) or inductive (6 short days - SD + 9 LD) photoperiodic conditions. The length of hypocotyl and cotyledon blade were measured and percentage of flowering was scored. Gibberellic acid (GA_3) stimulated hypocotyl growth of green and photobleached plants under SD and inhibited under LD conditions. Indole-3-acetic acid (IAA) slightly stimulated hypocotyl growth of green plants only under LD conditions. Benzylaminopurine (BAP) inhibited hypocotyl growth regardless of photoperiodic regime. The optimal concentration of glucose or saccharose for flowering in green and SANDOZ-treated plants was 5 %. In green SAN 9785-treated plants exogenous saccharides compensated lack of photosynthates to bring about full flowering, but SAN 9789-treated plants needed in addition GA_3.

Key words: benzylaminopurine, gibberellic acid, glucose, indole-3-acetic acid, saccharose, SANDOZ-herbicides.

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Abbreviations: BAP - benzylaminopurine; GA_3 - gibberellic acid; IAA - indole-3-acetic acid; LD(P) - long day(plant); SD(P) - short day(plant); SAN 9785 - 4-chloro-5-(dimethylamino)-2-phenyl-3(2H)-pyridazinone; SAN 9789 - 4-chloro-5(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone.

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Introduction

Various ecotypes of the short-day plant *Chenopodium rubrum* L., have been studied in different laboratories, interested in photoperiodic induction of flowering (Krekule *et al.* 1989, Seidlová *et al.* 1990, Crespi *et al.* 1993). *In vitro* grown *Chenopodium rubrum* L., ecotype 184, is a suitable model for studying photoperiodic and hormonal requirements for growth and flowering in the presence of SANDOZ-herbicides. Herbicide SAN 9789 inhibits the biosynthesis of carotenoids, leading to light-dependent oxidation of chlorophyll (Laskay and Lehoczki 1986, Wejnar and Appenroth 1990). When applied to the roots, SAN 9789 produces white photobleached plants. The other type of herbicide SAN 9785 inhibits the formation of chloroplast membrane polar lipids and also causes considerable decrease of photosynthetic activity and flowering in otherwise green leaves (Laskay *et al.* 1986).

According to the photomorphogenic phenomena (Jaben and Deitzer 1979, Gorton and Briggs 1980, Ćulafić *et al.* 1983, Heyde and Rombach 1988) the photoperiodic stimulus was perceived in the presence of SAN 9789, while the percentage of flowering was decreased (Živanović and Ćulafić 1992). As the flowering inhibition was not reversed with organic carbon sources (glucose or saccharose), it may be supposed that SAN 9789 also affects other factors, required for full flowering.

The aim of the present study has been to establish the role of saccharides and phytohormones in realization of perceived photoperiodic induction of flowering of *Chenopodium rubrum* L. plant.

Materials and methods

Plant material: The experiments were carried out with intact *Chenopodium rubrum* L. plants, ecotype 184. This ecotype comprises qualitative short-day plant, that flowers only when given at least five inductive cycles, with darkness longer than 8 h per day. Four cycles do not induce flowering, while five cycles induce flowering in more than 95 % of plants. The seeds were the gift of Prof. Dr. E. Wagner, originally obtained from Prof. B. Cumming's Laboratory. Seeds were surface-sterilized and aseptically sown on filter paper moistened with sterile water in Petri dishes. Uniform germination of the seeds was attained by temperature cycles and dark/light cycles as previously described (Živanović *et al.* 1988, 1992).

***In vitro* culture:** The seedlings were aseptically transferred into the culture tubes (five plants per tube and ten tubes per treatment). Each tube contained 10 cm^3 of basal medium (pH 5.5) which was supplemented with various hormones (0.1 - 10.0 mg dm^{-3} IAA or GA_3 or BAP), SANDOZ-herbicides (10.0 - 50.0 μM) and saccharides (2 - 7 % glucose or saccharose). The plants were kept under non-inductive conditions (18 to 6 h day/night cycle) in a phytotron (temperature 25 °C, relative humidity 70 %, irradiance 54 μmol m^{-2} s^{-1}).

Photoperiodic treatment: Five days after sowing, the plants were exposed to two photoperiodic regimes: non-inductive (15LD), consisting of 15 cycles of 18 h days,

or inductive (6SD + 9LD), consisting of 6 short days (14 h) followed by 9 long days. After the photoperiodic treatment the length of hypocotyl and cotyledon blade were measured and percentage of flowering was scored. The significance of differences between various treatments was evaluated by means of PC program *Statgraph* (one-way analysis of variance). Flowering was scored by using *Stereozoomicroscope* (*Baush & Lomb*, Rochester, USA). A fully developed flower was taken as the criterion for flowering.

Results and discussion

The effect of hormones on growth and flowering: Regardless of photoperiodic conditions, IAA (0.1 - 10.0 mg dm^{-3}) did not stimulate hypocotyl and cotyledon growth, the highest concentration being slightly inhibitory. The effect of BAP on hypocotyl and cotyledon growth was insignificant or slightly inhibitory. Auxins and cytokinins are usually considered to be the main components of the regulation of apical dominance (Krekule 1979). GA_3 (1.0 - 10.0 mg dm^{-3}) stimulated hypocotyl growth (Table 1). Cotyledon growth was not affected. As previously described by

Table 1. Effect of GA_3 on hypocotyl length [mm ± SE] of green and SAN 9789-treated plants grown under non-inductive or inductive conditions (% of control in parentheses).

	GA_3 [mg dm^{-3}]	Non-inductive conditions		Inductive conditions	
Green plants	0.0	4.72 ± 0.20	(100)	3.51 ± 0.11	(100)
	1.0	4.15 ± 0.24	(88)	4.21 ± 0.42	(120)
	5.0	6.60 ± 0.43	(140)	7.24 ± 0.37	(206)
	10.0	5.34 ± 0.29	(113)	7.64 ± 0.29	(218)
SAN 9789	0.0	2.76 ± 0.13	(100)	3.08 ± 0.06	(100)
	1.0	4.58 ± 0.13	(166)	5.37 ± 0.16	(174)
	5.0	4.48 ± 0.19	(162)	5.37 ± 0.21	(174)
	10.0	5.35 ± 0.20	(194)	5.42 ± 0.17	(176)

Seidlová (1989), GA_3 stimulated shoot elongation, irrespective of the photoperiodic treatments, and branching of shoot apex in induced *Chenopodium* plants. IAA and BAP (0.1 - 10.0 mg dm^{-3}) inhibited flowering in green plants (Table 2 and Fig. 1). In SAN 9789-treated plants, flowering was inhibited at higher IAA and BAP concentrations (1.0 - 10.0 mg dm^{-3}). Exogenously applied IAA has inhibitory and promotive effect on flowering in SDPs and LDPs (Jacobs 1985, Bernier *et al.* 1981). The inhibitory effect of auxin on flowering in *Chenopodium rubrum* L. was indeed found to rely on the increasing apical dominance at the apex (Seidlová and Khatoon 1976). According to *in vitro* studies auxins did not simply oppose flowering (Bernier *et al.* 1981, Bernier 1988). GA_3 (0.1 - 10.0 mg dm^{-3}) stimulated flowering up to 100 % in SAN 9789-treated plants under inductive regime (Table 2). GA_3 cannot substitute for photoinduction in the majority of SDPs under constant unfavourable light conditions. Thus in *Chenopodium rubrum* L. GA_3 was not capable of inducing

flower formation in strongly non-inductive conditions (Seidlová 1985). In *Pharbitis nil* (SDP) GA_3 may be promotive for flowering when applied before the inductive dark period, although inhibitory when applied after it (King *et al.* 1987). On the

Table 2. Percentage of flowering in green and SAN 9789-treated plants, grown under inductive conditions, on basal medium supplemented with hormones (0.1 - 10.0 mg dm^{-3}).

Plants	Hormones	Concentration [mg dm^{-3}]				
		0.0	0.1	1.0	5.0	10.0
Green	None	100	-	-	-	-
SAN 9789		38	-	-	-	-
Green	IAA	-	69	0	0	0
SAN 9789		-	52	0	0	0
Green	BAP	-	62	0	0	0
SAN 9789		-	38	0	0	0
Green	GA_3	-	100	100	100	100
SAN 9789		-	100	100	100	100

- not measured

other hand, GA_3 and other gibberellins have been shown to promote the switch from vegetative growth to flowering in some LDPs or cold-requiring plants under non-inductive conditions (Wilson *et al.* 1992). GA_3 was found to stimulate markedly stem elongation with little or no effect on flowering in *Lolium temulentum* L. (Evans 1994). Evans (1994) supposed different gibberellins receptors for stem elongation and early events in flowering process at the shoot apex. Under non-inductive conditions no flowering was observed either in control or hormones-treated plants in our experimental conditions. The existence of great diversity in results with GA_3 could be explained by various sensitivity of different species to different gibberellins applied at appropriate time and growing conditions.

The effect of glucose and saccharose on growth and flowering: Green and SAN 9789-treated plants were grown *in vitro* on a medium that contained increasing concentrations (2 - 7 %) of glucose or saccharose under non-inductive or inductive conditions (Table 3). Saccharides (either glucose or saccharose) inhibited the growth of hypocotyl both in green and SAN 9789-treated plants, regardless of photoperiodic conditions. The effect of saccharides on cotyledons of SAN 9789-treated plants was stimulative.

In green plants, grown without saccharides, flowering was recorded in 81 %. When plants were supplemented with 3 - 7 % glucose or saccharose they 100 % flowered. SAN 9789-treated plants did not flower on 2 % and 3 % saccharides, while at 5 % glucose and 7 % saccharose 20 - 30 % of plants flowered. The participation of saccharides in the control of flowering has been shown in *Sinapis alba* L. (Lejeune *et al.* 1991), *Lolium temulentum* (McDaniel 1991) and *Pharbitis nil* (Ishioka *et al.* 1991).

Table 3. Effect of saccharides on hypocotyl length [mm ± SE] of green and SAN 9789-treated plants grown under non-inductive and inductive conditions (% of control in parentheses). - not measured.

Plants	Saccharides	Concentration [%] 0	2	3	5	7
Non-inductive conditions						
Green	none	8.29 ± 0.29	-	-	-	-
Green	glucose	-	5.03 ± 0.16 (61)	4.44 ± 0.14 (54)	3.00 ± 0.10 (36)	3.10 ± 0.13 (37)
SAN 9789	glucose	-	3.00 ± 0.12	2.88 ± 0.14	2.97 ± 0.14	2.38 ± 0.12
Green	saccharose	-	5.35 ± 0.26 (64)	4.60 ± 0.40 (55)	4.25 ± 0.16 (51)	4.02 ± 0.14 (48)
SAN 9789	saccharose	-	2.9 ± 0.13	3.07 ± 0.48	3.33 ± 0.15	3.42 ± 0.20
Inductive conditions						
Green	none	7.19 ± 0.35	-	-	-	-
Green	glucose	-	5.50 ± 0.24 (76)	5.87 ± 0.21 (82)	4.84 ± 0.12 (67)	4.08 ± 0.15 (57)
SAN 9789	glucose	-	3.65 ± 0.16	3.31 ± 0.15	2.94 ± 0.13	2.67 ± 0.13
Green	saccharose	-	5.68 ± 0.18 (79)	5.28 ± 0.19 (73)	4.78 ± 0.19 (66)	4.47 ± 0.19 (62)
SAN 9789	saccharose	-	3.28 ± 0.20	4.29 ± 0.14	2.79 ± 0.20	3.21 ± 0.08

Comparison of SAN 9785 and SAN 9789 effects: SAN 9785- and SAN 9789-treated plants did not flower without 5 % glucose. When glucose was added, we found clear differences between the effects of SAN 9785 and SAN 9789 on hypocotyl growth and flowering, but not on cotyledon growth (Table 4). SAN 9789 had inhibitory effect (Fig. 1), while SAN 9785 in the same concentration (10 μM) did not affect growth and flowering. In the presence of 5 % glucose even higher concentration of SAN 9785 was not inhibitory. Exogenous saccharides fully compensate for the loss of photosynthates required for flowering in SAN 9785-treated plants. In SAN 9789-treated, photobleached plants, exogenous saccharide are insufficient for full flowering. However, the addition of GA_3 compensated for other factors necessary for

Table 4. Effect of SAN 9785 and SAN 9789 on hypocotyl and cotyledon length (% of control in parentheses) and flowering of plants grown under inductive conditions.

Herbicide	Concentration [μM]	Hypocotyl length [mm ± SE]	Cotyledon length [mm ± SE]	Flowering [%]
Control	0.0	6.19 ± 0.19 (100)	2.69 ± 0.05 (100)	100
SAN 9785	10.0	5.33 ± 0.20 (86)	2.46 ± 0.13 (91)	100
	50.0	5.56 ± 0.27 (90)	2.38 ± 0.08 (88)	100
SAN 9789	10.0	3.10 ± 0.06 (50)*	2.46 ± 0.13 (91)	38

*Significant inhibition over control at $P = 0.05$.

100 % flowering response. Both SANDOZ herbicides did not affect cotyledon growth. There is evidence that the chloroplasts represent the main cell compartment for gibberellin synthesis and metabolism (Hilton and Smith 1980). As this function of chloroplasts were in some extension impaired by SAN 9789, the flowering could be inhibited.

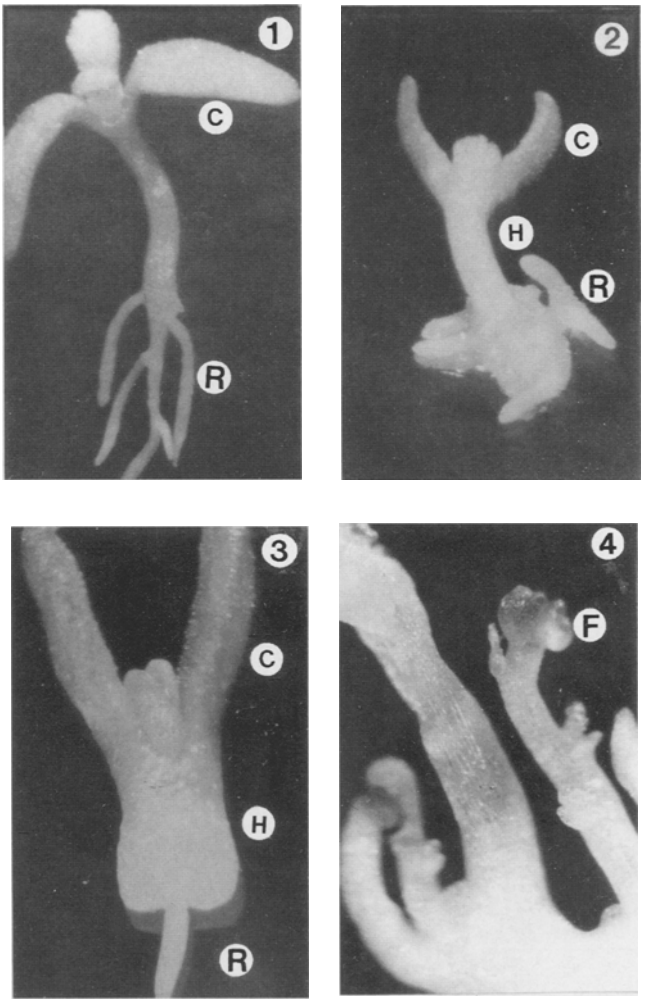

Fig. 1. Effects of IAA, BAP and GA_3 on growth and flowering in 10 μM SAN 9789-treated 21-d-old plants grown under inductive conditions. *1* - control (vegetative plant); *2* - 5.0 mg dm^{-3} IAA (vegetative plant); *3* - 5.0 mg dm^{-3} BAP (vegetative plant); *4* - 5.0 mg dm^{-3} GA_3 (flowering plant). *C* - cotyledon; *H* - hypocotyl; *R* - roots; *F* - flower.

In conclusion, our results show the close relationship between the growth of vegetative organs and photoperiodic induction of flowering. Hypocotyl growth was transiently inhibited in green plants under inductive conditions. GA_3 showed to be

the most effective hormone involved in flowering. It would be very interesting to test this relationship for the same and additional factors in *Chenopodium murale* L. (LDP) that influence photoperiodic induction of flowering.

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