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BRIEF COMMUNICATION

The effects of growth regulators on flowering of *Chenopodium murale* plants *in vitro*

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Abstract

In vitro culture of *Chenopodium murale* L. (ecotype 197) green and herbicide SAN 9789 - treated "white" plants was established and the effects of benzylaminopurine (BAP), indole-3-acetic acid (IAA) and gibberellic acid (GA_3) on growth and flowering were tested. Green plants did not flower on glucose free media, while 17 % of plants flowered on 5 % glucose-containing medium. SAN 9789 (10^{-5} M) inhibited growth and flowering. BAP and IAA (0.1 - 5 mg dm^{-3}) also inhibited growth and flowering of green and "white" plants. GA_3 (10 mg dm^{-3}) stimulated leaf development in green plants, but had no significant effect on "white" plants, and stimulated flowering of green (41 %) and "white" (33 %) plants.

Additional key words: benzylaminopurine, gibberellic acid, herbicide SAN 9789, indole-3-acetic acid.

Chenopodium murale (long-day plant) as well as previously investigated *C. rubrum* (short-day plant) are suitable objects for studying photoperiodic and hormonal requirements for growth and flowering (Živanović *et al.* 1995). The herbicide SAN 9789 produces "white" (photobleached) plants (Živanović and Ćulafić 1992). In the presence of SAN 9789 the photoperiodic stimulus was perceived, although the percentage of flowering was decreased in long-day *Spinacia oleracea* (Ćulafić *et al.* 1983) and short-day *C. rubrum* (Živanović *et al.* 1995) plants. As the flowering inhibition was not possible to be completely reversed by the organic carbon sources, it was supposed that SAN 9789 also affects phytohormones required for flowering (Živanović *et al.* 1995). In *C. rubrum* auxin application mostly inhibited flowering. The effect strongly depended on timing with respect to photoperiodic induction. However, the concentration of auxin applied on *C. rubrum* was at least two orders of magnitude above the fluctuation of endogenous auxins (Pavlová and Krekule 1984, Krekule *et al.* 1985). Podolny *et al.* (1991) suggested that IAA took place in both perception and realization of photoperiodic signal. Auxins are also inhibitors of flowering *in vitro* (for review see Scorza 1982). Cytokinins are considered as one component of the floral stimulus (Bernier *et al.* 1977). Promotion as well as inhibition of flowering was obtained in various plants after application of cytokinins. Cytokinins did not compensate for unsuitable photoperiodic conditions for flowering in *Sinapis alba*, but could initiate some early events of the floral program (Havelange *et al.* 1986). Chailakhyan (1958) suggested gibberellins as a part of a specific hormone florigen. Under conditions of threshold induction, GA_3 had slightly promoting effect on flowering of *C. rubrum* (Seidlová 1985). *C. rubrum* (Živanović *et al.* 1995) and *C. murale* (Mitrović *et al.* 2000) could not be induced to flowering by GA_3 under non-inductive conditions, but under inductive conditions, GA_3 stimulated flowering.

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Abbreviations: BAP - benzylaminopurine, GA_3 - gibberellic acid, IAA - indole-3-acetic acid, SAN 9789 - 4-chloro-5(methylamino)-2-(α, α, α-trifluoro-m-tolyl)-3(2H)pyridazinone.

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The aim of this study was to investigate the effects of growth regulators (IAA, BAP and GA_3) and herbicide SAN 9789 on growth and flowering *in vitro* of *Chenopodium murale* ecotype 197 in comparison with previously published data for *C. rubrum* (Živanović *et al.* 1995).

The experiments were carried out with intact *Chenopodium murale* L. plants, ecotype 197, derived from seeds sown *in vitro*. Surface sterilized seeds (Mitrović 1998) were aseptically sown on filter paper in Petri-dishes moistened with sterile distilled water (green plants) or with 10^{-5} M SAN 9789 ("white" plants). Five-day-old green seedlings or 7-d-old "white" seedlings were aseptically transferred into Erlenmeyer flasks (8 - 10 seedlings per flask), that contained 50 cm^3 of modified Hoagland's mineral solution (Wagner and Leonhard 1985), supplemented with glucose (5 %), agar (0.62 %) and/or SAN 9789 (10^{-5} M), and/or IAA (0.1 - 5.0 mg dm^{-3}), BAP (0.1 - 5.0 mg dm^{-3}), and GA_3 (10.0 mg dm^{-3}). Media were autoclaved for 25 min at 114 °C and 90 kPa; pH was adjusted to 5.7. The seedlings were exposed to non-inductive conditions (short days): 16 h of darkness + 8 h of light (irradiance of 70 μmol m^{-2} s^{-1}) provided by 4 fluorescent tubes (4 × 18 W, *Osram*, FRG) in combination with 2 incandescent tubes (2 × 60 W, *Linestra-Osram*), until development of 1st pair of leaves and then transferred to inductive conditions for 10 d (continuous light, 2 incandescent tubes,1.9 μmol m^{-2} s^{-1}). Following induction the plants were transferred back to non-inductive conditions for 30 d. The plants were grown on two-phase media (fresh liquid media were added every 10 - 15 d on solidified agar medium). Each experiment was repeated twice. Hypocotyl and the 1st internode length were measured, number of leaves was determined and percentage of flowering was scored using stereomicroscope (*Baush and Lomb*, Rochester, USA). A fully developed flower was taken as a criterion for flowering. The significance of difference between various treatments was evaluated by means of PC program *Statgraphics* (one-way analysis of variance).

Green *C. murale* plants grown on glucose free medium did not flower, while 17 % of plants flowered on 5 % glucose-containing medium. SAN 9789-treated ("white") plants did not survive on glucose free media. SAN 9789 (10^{-5} M) inhibited hypocotyl growth, which was also reported for *C. rubrum* (Živanović and Ćulafić 1992), and leaf development (Table 1). Flowering inhibition of "white" *C. murale* plants (Table 1) was also reported for "white" *C. rubrum* plants *in vitro* (Živanović *et al.* 1995). On the other hand, SAN 9789 only slightly inhibited flowering of isolated shoots of *Spinacia oleracea in vitro* (Ćulafić *et al.* 1983).

Table 1. The effects of IAA, BAP and GA_3 on growth and flowering of green and "white" *C. murale* plants induced at the phase of the 1st pair of leaves by 10 d of continuous light and evaluated 30 d after the induction; means ± SE, $n = 20$, * - significant difference against respective control at $P = 5$ %.

Plants	Growth regulators	[mg dm^{-3}]	Glucose [%]	Hypocotyl length [mm]	Number of leaves	Flowering [%]
Green	none		0	22.77 ± 0.76	5.87 ± 0.16	0
	none		5	15.48 ± 0.27	7.28 ± 0.13	17
	IAA	0.1	0	21.64 ± 1.16*	6.00 ± 0.21	0
		1.0	0	21.67 ± 0.54*	6.11 ± 0.11*	0
		5.0	0	15.79 ± 0.73*	6.14 ± 0.14*	0
		0.1	5	12.64 ± 0.70*	6.72 ± 0.30	9
		1.0	5	13.27 ± 0.61*	6.27 ± 0.43*	7
		5.0	5	9.43 ± 0.78*	6.00 ± 0.62	0
	BAP	0.1	0	13.82 ± 0.78*	5.09 ± 0.31	0
		1.0	0	10.33 ± 0.59*	4.33 ± 0.22*	0
		5.0	0	8.00 ± 0.71*	2.40 ± 0.27*	0
	GA_3	10.0	0	21.19 ± 1.23	6.13 ± 0.34	0
		10.0	5	13.31 ± 0.57*	8.83 ± 0.35*	41
"White"	none		5	6.38 ± 0.35	4.66 + 0.35	0
	IAA	0.1	5	5.13 ± 0.55	4.25 ± 0.25	0
		1.0	5	3.07 ± 0.43*	4.50 ± 0.37	0
		5.0	5	3.80 ± 0.26*	3.47 ± 0.31	0
	BAP	0.1	5	4.52 ± 0.23*	2.80 ± 0.22*	0
		1.0	5	3.82 ± 0.15*	3.00 ± 0.16*	0
		5.0	5	2.48 ± 0.13*	4.47 ± 0.18	0
	GA_3	10.0	5	17.05 ± 0.86*	4.33 ± 0.18	33

IAA (0.1 - 5.0 mg dm^{-3}) inhibited hypocotyl growth and had no significant effect on leaf development both in green and "white" *C. murale* plants (Table 1). IAA (0.1 - 1 mg dm^{-3}) also inhibited flowering in green *C. murale* plants, lowering the percentage of flowering to 7 %, as compared to control (17 %). Neither control nor IAA-treated "white" *C. murale* plants did flower (Table 1). IAA also inhibited growth and flowering of green and "white" *C. rubrum* plants (Živanović *et al.* 1995).

BAP (0.1 - 5.0 mg dm^{-3}) inhibited hypocotyl growth and leaf development both in green and "white" plants (Table 1). Green or "white" plants did not flower on BAP-containing media. BAP also inhibited growth and flowering *in vitro* of green and "white" *C. rubrum* plants (Živanović *et al.* 1995) under inductive conditions, and flowering of apical segments *in vivo* (Vondráková 1992), but promoted flowering of intact *C. rubrum* plants when applied at the beginning of the inductive darkness (Vondráková *et al.* 1998).

Herbicide SAN 9789 caused the destruction of chloroplasts as the place of synthesis and metabolism of gibberellins (Rappaport and Adams 1979, Hilton and Smith 1980). We supposed that the addition of GA_3 might compensate the lack of synthesis of gibberellins in "white" *C. murale* plants. GA_3 (10 mg dm^{-3}) inhibited hypocotyl growth of green plants grown on 5 % glucose-containing media and stimulated hypocotyl growth of "white" *C. murale* plants, as was also reported for *C. rubrum* plants (Živanović *et al.* 1995). On the other hand, GA_3 stimulated leaf development in green, while it had no significant effect on leaf development in "white" *C. murale* plants (Table 1). The results obtained in *Lolium temulentum* (Evans *et al.* 1994) indicated the existence of different gibberellin-receptors in stem apex for stem elongation and early events in flowering. GA_3 stimulated flowering of green and "white" *C. murale* plants (Table 1). The addition of 10 mg dm^{-3} GA_3 on 5 % glucose-containing medium resulted in 41 % of flowering in green plants in comparison to 17 % flowering of controls. Control "white" plants did not flower, while 33 % of "white" plants flowered on 10 mg dm^{-3} GA_3-containing media (Table 1). This data could be in relation to the Živanović and Ćulafić (1992) assumption that the inhibitory effect of SAN 9789 on flowering was due to the overall inhibition of growth. In *Spinacia oleracea* flowering was followed by the stem elongation and SAN 9789 inhibits this process. "White" *S. oleracia* plants flowered under non-inductive conditions on GA_3-containing media (Ćulafić *et al.* 1983). GA_3 stimulated flowering of "white" *C. rubrum* plants (Živanović *et al.* 1995).

In general, our results indicate that the effects of phytohormones on growth and flowering of green and "white" *C. murale* plants are similar to those obtained for *C. rubrum* (Živanović *et al.* 1995). As they are the plants with different photoperiodic requirements, this might suggest that the similar unique hormonal signals regulate the transition from vegetative to reproductive phase of development.

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