

The effects of photoperiod, glucose and gibberellic acid on growth *in vitro* and flowering of *Chenopodium murale*

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Abstract

In vitro culture of long-day plant *Chenopodium murale* L. was established. The effects of photoperiod, glucose and gibberellic acid (GA₃) on flowering and growth *in vitro* were investigated. Oscillatory changes of photoperiodic sensitivity were noticeable with regard to plant age. The plants induced at the phase of the 1st and the 3rd pair of leaves flowered to higher degree than those induced at the phase of 2nd pair. Plants induced at the phase of the 1st pair of leaves flowered to 17 % on 5 % glucose-containing medium and the addition of 5 mg dm⁻³ GA₃ resulted in maximum flowering (43 %). Neither glucose nor GA₃ were able to compensate for photoperiodic requirements for flowering. Hypocotyl growth was decreased and the 1st internode elongation and development of leaves were increased due to inductive photoperiodic conditions, as compared to non-inductive ones.

Additional key words: hypocotyl elongation, 1st internode elongation, leaf initiation, photoperiodic sensitivity.

Introduction

Chenopodium murale L. is a herbaceous non-rosette annual. Ecotype 197 is a facultative long-day plant (Cumming 1967). The use of long-day species of genus *Chenopodium* of a similar type of morphogenesis, as short-day ones, may be important for comparative studies of flowering and its regulation (Pavlová *et al.* 1989b). *C. murale* L. is sensitive to induction of flowering as early as at the phase of the first pair of leaves (Pavlová *et al.* 1989a). Oscillatory changes of photoperiodic sensitivity were noticeable between 2nd and 6th pair of leaves. Growth activation of *C. murale in vivo* was associated with transfer to inductive light treatment (Pavlová *et al.* 1989b). On the contrary, in *C. rubrum* the inductive short days brought about the growth inhibition, which has been followed by a rise of growth rate (Ullmann *et al.* 1980).

According to Scorza (1982) and Dickens and van Staden (1988), *in vitro* culture of intact plants (the plants derived from seed sown *in vitro*) might have an advantage over greenhouse grown plants, by precise control of

environmental factors and the application of growth regulators.

Saccharides (sucrose, glucose, maltose, lactose, raffinose) are necessary as carbon sources in culture media for reliable induction and development of flowers in different species, sucrose being the most commonly used (Nitsch 1972, Scorza 1982). Sucrose (1 - 3 %) was necessary in the culture media for reliable induction of isolated buds of *Spinacia oleracea* (Čulafić 1973). The interaction of sucrose and light to promote *in vitro* flowering has been reported in a number of species (Scorza 1982). In the present study we used glucose, relying on previous paper (Živanović *et al.* 1995), which showed no significant difference between effect of glucose and sucrose on growth and flowering of intact *C. rubrum in vitro*. Also, glucose showed significant effect on floral morphogenesis in shoot apical meristems of *Pharbitis nil* following their culture at various times after induction (Durdan *et al.* 1998).

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Exogenously applied gibberellins (GA₃) induce or promote flowering in many species, being particularly effective in long-day plants, *e.g.* in *Lolium temulentum* (Pharis *et al.* 1987, Evans *et al.* 1994), or in *Spinacia oleracea* (Čulafić 1973). Lang (1965) supposed that GA₃ affected flower development rather than flower initiation. Seidlová (1989) suggested that the effect of GA₃ depended on the developmental stages of bud primordia, as targets for hormone action. Flower-promoting or flower-inhibiting effect of GA₃ depended also on its

concentration (Seidlová 1989). Evans *et al.* (1990) showed in the long-day plant *Lolium temulentum* that different GA₃ promoted both stem elongation and inflorescence initiation to different extent.

The aim of this study was to establish *in vitro* culture of *C. murale* to investigate the effects of glucose and GA₃ on growth and flowering and to find out how the photoperiodic treatment, leading to flowering, is correlated with the growth of vegetative organs.

Materials and methods

Plants: The experiments were carried out with intact *Chenopodium murale* L. plants, ecotype 197, derived from seeds sown *in vitro*. The seeds were a gift of the Institute of Experimental Botany of Academy of Sciences of Czech Republic in Prague, and were further propagated in the greenhouse of the Institute for Biological Research "Siniša Stanković" in Belgrade. Seeds were surface sterilized with 4 % Na-hypochlorite for 10 min, washed with sterile distilled water and aseptically sown on moistened filter paper in Petri dishes. Uniform germination of seeds was attained by temperature and dark/light cycles, as previously described for *C. rubrum* (Živanović and Čulafić 1992). Five-day-old seedlings were aseptically transferred into Erlenmeyer flasks that contained 50 cm³ of modified Hoagland's mineral solution as basal culture medium (Wagner and Leonhard 1985), gelled with 0.62 % agar, and supplemented with glucose (2 - 10 %) and/or GA₃ (0.1 - 10 mg dm⁻³).

Photoperiodic treatments: Seedlings, with fully developed cotyledons (5-d-old) were exposed to different photoperiodic conditions (non-inductive or inductive). Control plants were grown under non-inductive conditions (45 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*, Germany) in combination with 2 incandescent tubes (2 × 60 W, *Linestra-Osram*). The plants were grown under non-inductive conditions until

the 1st pair of leaves was developed (8 - 12 d) and then transferred to inductive conditions, *i.e.* 10 d of continuous light of 2 incandescent tubes (1.9 μmol m⁻² s⁻¹). Following induction the plants were transferred back to non-inductive conditions for 23 - 27 d. One group of experiments was designed for studying the age sensitivity of plants to photoperiod. The plants were kept under non-inductive conditions from 9 to 29 or from 19 to 42 d to develop the 2nd and the 3rd leaf pair, respectively, depending on media composition. After the induction, the plants were grown under non-inductive conditions for another 30 d. The plants were grown on two-phase media (fresh liquid media were added every 10 - 15 d on media solidified with agar).

Measurements and statistics: Each experiment comprised two Erlenmeyer flasks per medium and 8 - 10 plants per each flask. Each experiment was repeated twice. After the photoperiodic treatment, hypocotyl and the 1st internode length were measured, the number of leaves was determined and percentage of flowering was scored. The significance of differences between various treatments was evaluated by means of PC program *Statgraphics* (one-way analysis of variance). Flowering was scored using stereomicroscope (*Bausch & Lomb*, Rochester, USA). A fully developed terminal flower was taken as a criterion for flowering.

Results and discussion

Age-dependent sensitivity of plants to photoperiod: The plants were grown *in vitro* under non-inductive conditions until the 1 - 3rd pair of leaves was developed. This period (up to 42 d) was much longer than that in experiments *in vivo* (Pavlová 1989b). We used leaves, which attained about half of their final length according to Khudairi and Hamner (1954), as a criterion for transferring the plants to inductive conditions. Plants were grown on glucose-free media (control) and 5 % glucose-

containing media, supplemented with GA₃. There was no flowering on glucose-free medium, regardless of the phase of development (Tables 1, 2). The addition of GA₃ (1 or 5 mg dm⁻³) to glucose-free medium had no effect on flowering, when the plants were induced at the phase of the 1st (Table 1) and the 2nd (Table 2) pair of leaves. When the plants were induced at the phase of the 3rd pair of leaves, the addition of GA₃ (1 or 5 mg dm⁻³) resulted in 33 or 10 % flowering, respectively (Table 2). These data

might be explained by existence of age-dependent control of flowering (Chailakhyan 1988).

It is evident that the plants induced at the phase of the 1st (Table 1) or 3rd (Table 2) pair of leaves flowered almost to the same extent (17 or 18 %) on 5 % glucose-containing media, but when the plants were induced at the phase of the 2nd pair of leaves (Table 2), there was no flowering. The addition of GA₃ (1 or 5 mg dm⁻³) to 5 % glucose-containing medium, increased percentage of flowering up to 40 or 43 %, respectively, when the plants were induced in the phase of 1st pair of leaves (Table 1).

On the same media, plants induced at the phase of 2nd pair of leaves flowered to a lower percentage (19 or 13 %, respectively), but the plants induced at the phase of 3rd pair of leaves, flowered up to 50 or 67 %, respectively (Table 2). Pavlová *et al.* (1989b) reported the similar low percentage of flowering *in vivo* of *C. murale*, induced at the phase of the 2nd pair of leaves.

Detailed investigations of the effect of glucose (2 - 5 %) and GA₃ (0.1 - 10 mg dm⁻³) on flowering and growth were performed on *C. murale* plants induced at the phase of the 1st pair of leaves (Table 1).

Table 1. Effects of glucose [%] and GA₃ [mg dm⁻³] on growth and flowering of *C. murale* grown under non-inductive (NI; 45 SD) and inductive (I; 8 - 12 SD + 10 DCL; 23 - 27 SD) photoperiodic conditions (plants induced at the phase of the 1st pair of leaves); DCL - days of continuous light. SD - short day (8/16 h light/darkness); means \pm SE, n = 20, * - significant against respective control at *P* = 5 %.

Treatment	Glucose	GA ₃	Hypocotyl length [mm]		1st internode length [mm]		Number of leaves		Flowering [%]	
			NI	I	NI	I	NI	I	NI	I
0	0.0		26.73 \pm 0.74	22.77 \pm 0.76	0.13 \pm 0.09	5.87 \pm 0.17	2.67 \pm 0.25	5.87 \pm 0.16	0	0
	0.1	-	-	29.67 \pm 0.68*	-	7.67 \pm 0.84	-	5.44 \pm 0.22	0	0
	1.0		34.10 \pm 1.24*	22.36 \pm 1.26	1.10 \pm 0.31	15.43 \pm 0.84*	2.20 \pm 0.20	7.14 \pm 0.27*	0	0
	5.0		29.55 \pm 0.85	25.27 \pm 0.66	6.27 \pm 0.54*	14.27 \pm 1.00*	4.91 \pm 0.31*	6.67 \pm 0.25	0	0
	10.0		34.50 \pm 1.03*	21.19 \pm 1.23	4.00 \pm 0.39*	15.81 \pm 1.07*	3.83 \pm 0.17*	6.13 \pm 0.34	0	0
2	0.0		27.64 \pm 0.89	23.41 \pm 0.70	2.59 \pm 0.21	6.00 \pm 0.35	4.91 \pm 0.22*	6.00 \pm 0.00	0	0
	0.1	-	-	26.18 \pm 0.94*	-	7.41 \pm 0.42	-	6.71 \pm 0.29	0	0
	1.0		31.64 \pm 1.03*	20.31 \pm 0.62	12.00 \pm 0.94*	12.23 \pm 0.54*	6.14 \pm 0.33*	7.38 \pm 0.27*	0	0
	5.0		36.18 \pm 0.98*	23.68 \pm 0.84	10.05 \pm 0.91*	16.05 \pm 0.83*	5.91 \pm 0.15	7.47 \pm 0.26*	0	0
	10.0		30.95 \pm 0.94	18.42 \pm 1.18*	12.70 \pm 1.09*	14.08 \pm 0.70*	5.41 \pm 0.26	7.50 \pm 0.44*	0	8
3	0.0		24.30 \pm 0.83	19.95 \pm 0.75*	2.15 \pm 0.30	6.68 \pm 0.45	4.90 \pm 0.23*	6.11 \pm 0.11	0	0
	0.1	-	-	22.06 \pm 0.75	-	6.65 \pm 0.38	-	6.12 \pm 0.32	0	0
	1.0		34.13 \pm 0.95*	15.06 \pm 0.65*	9.31 \pm 0.69*	9.38 \pm 0.91*	5.25 \pm 0.36	6.88 \pm 0.36	0	13
	5.0		29.56 \pm 0.89*	21.60 \pm 0.69	14.44 \pm 1.12*	16.20 \pm 0.50*	6.44 \pm 0.29*	8.27 \pm 0.27*	0	13
	10.0		27.59 \pm 0.84	16.27 \pm 0.60*	15.94 \pm 0.91*	12.20 \pm 1.06*	6.35 \pm 0.26*	7.20 \pm 0.51*	0	27
5	0.0		17.85 \pm 0.95*	15.48 \pm 0.27*	1.85 \pm 0.91	4.49 \pm 0.17	5.85 \pm 0.36*	7.28 \pm 0.13*	0	17
	0.1	-	-	14.89 \pm 0.86	-	4.78 \pm 0.60	-	7.56 \pm 0.29	0	22
	1.0		23.00 \pm 0.80*	14.40 \pm 0.98	10.56 \pm 0.58*	6.20 \pm 0.86	6.44 \pm 0.56	9.20 \pm 0.80*	0	40
	5.0		22.63 \pm 1.28*	15.49 \pm 0.42	12.63 \pm 1.22*	10.78 \pm 0.38*	6.50 \pm 0.33	8.67 \pm 0.28*	0	43
	10.0		29.29 \pm 1.84*	13.31 \pm 0.57*	14.71 \pm 0.97*	10.90 \pm 0.51*	7.43 \pm 0.37*	8.83 \pm 0.35*	0	41

The effects of glucose and GA₃ on flowering: The plants grown under inductive conditions, did not flower either on the glucose-free media or on the media with lower content of glucose (2 or 3 %). The addition of 5 % glucose resulted in 17 % of flowering (Table 1), as also previously described (Mitrović *et al.* 2000). Preliminary experiments showed that higher glucose concentrations (7 or 10 %) were deleterious and that is why we did not use them for further studies (Mitrović 1998).

The addition of GA₃ (10 mg dm⁻³) to 2 or 3 % glucose-containing medium, resulted in 8 or 27 % of flowering, respectively (Table 1). This might be attributed to cumulative effect of glucose and GA₃ on flowering.

Maximum flowering (43 %) was obtained by the addition of 5 mg dm⁻³ GA₃ on 5 % glucose-containing medium (Table 1), which could be explained as a promotive effect of GA₃ on flower development according to Seidlová (1989). Neither glucose nor GA₃ were able to compensate for photoperiodic requirement for flowering of *C. murale* under non-inductive conditions (Table 1), which was also reported for *C. rubrum* (Seidlová 1985, Živanović *et al.* 1995). According to previously reported results (Pavlová *et al.* 1989b) from experiments *in vivo*, 100 % of plants flowered, when induced at the phase of the 1st pair of leaves by 5 d of continuous light, while under our inductive conditions (10 d of continuous light), maximum

flowering was 43 %. Thus our *in vitro* conditions might be limiting for flowering.

The effect of photoperiod on growth: Inductive photoperiodic treatment had an inhibitory effect on hypocotyl elongation of *C. murale* and it significantly stimulated the 1st internode elongation and increased number of leaves (Table 1) as compared to non-inductive conditions. Pavlová *et al.* (1989b) reported that the overall growth activation (hypocotyl, cotyledons and the 1st internode elongation) was associated apparently with transfer of *C. murale* plants to inductive light treatment *in vivo*. The transition to reproductive phase in *C. murale* strongly resembled that in other *Chenopodium* species: leaf initiation was accelerated and axillary meristems were activated, as previously described (Thomas 1961, Gifford and Tepper 1962, Cumming 1967). Inductive photoperiodic conditions stimulated the 1st internode elongation in short-day *C. rubrum* plants grown *in vivo* (Seidlová and Sádliková 1983) and brought about growth changes which might be integrated into the growth pattern of apical meristem essential for reproductive development (Krekule *et al.* 1989).

The effects of glucose and GA₃ on growth: Glucose (2 - 5 %) inhibited *C. murale* hypocotyl elongation, regardless of photoperiodic conditions (Table 1). Glucose did not have any significant effect on 1st internode

elongation, under inductive or non-inductive conditions (Table 1). Glucose increased the number of leaves both under inductive and non-inductive conditions (Table 1). According to the results, reported on *C. rubrum* (Živanović *et al.* 1995), saccharides (either glucose or sucrose) inhibited the hypocotyl growth, regardless of photoperiodic conditions similar to our own results on *C. murale*.

GA₃ (1 - 10 mg dm⁻³) did not affect hypocotyl elongation under inductive conditions (Table 1) while it had stimulatory effect under non-inductive conditions. GA₃ stimulated 1st internode elongation (Table 1), and slightly increased number of leaves (Table 1) both under inductive and non-inductive conditions. GA₃ had almost similar effect on growth of vegetative organs, regardless of glucose (2 - 5 %) concentration in the media (Table 1). According to previous results (Živanović *et al.* 1995, Seidlová *et al.* 1990), GA₃ had similar stimulatory effect on hypocotyl elongation of *C. rubrum* under non-inductive conditions and it stimulated 1st internode elongation under inductive ones. Also, exogenous application of GA₃ brought about stem elongation, by stimulation of cell division and cell elongation, independently of photoperiodic treatment (Seidlová 1989). Evans supposed different GA₅ receptors for stem elongation and early events in flowering process at the shoot apex (Evans *et al.* 1994).

Table 2. Effects of glucose [%] and GA₃ [mg dm⁻³] on growth and flowering of *C. murale* L. induced at the phase of 2nd (9 - 29 SD + 10 DCL + 30 SD) and 3rd (19 - 42 SD + 10 DCL + 30 SD) pair of leaves; DCL - days of continuous light. SD - short day (8/16 h light/darkness); means \pm SE, *n* = 20, * - significant against respective control at *P* = 5 %.

Treatment	Glucose GA ₃	Hypocotyl length [mm]		1 st internode length [mm]		Number of leaves		Flowering [%]	
		2 nd	3 rd	2 nd	3 rd	2 nd	3 rd	2 nd	3 rd
0	0.0	27.61 \pm 0.59	28.25 \pm 0.77	4.64 \pm 0.27	4.50 \pm 0.21	7.57 \pm 0.30	8.21 \pm 0.21	0	0
	1.0	29.85 \pm 1.25	31.42 \pm 1.64	9.54 \pm 0.93*	12.42 \pm 1.06*	7.38 \pm 0.27	9.33 \pm 0.28	0	33
	5.0	24.36 \pm 0.56*	25.55 \pm 1.34	24.36 \pm 0.56*	15.90 \pm 0.99*	7.73 \pm 0.30	9.00 \pm 0.31	0	10
5	0.0	20.70 \pm 0.99*	17.50 \pm 0.47*	3.90 \pm 0.40	4.50 \pm 0.81	6.20 \pm 0.20	6.50 \pm 0.26*	0	18
	1.0	19.06 \pm 0.66	19.50 \pm 1.01	8.49 \pm 0.77*	12.17 \pm 1.16*	8.50 \pm 0.77*	8.34 \pm 0.47*	19	50
	5.0	12.20 \pm 0.74*	19.00 \pm 1.53	7.20 \pm 1.21*	13.33 \pm 0.88*	4.66 \pm 0.47*	7.33 \pm 0.67	13	67

The effects of glucose (5 %) and GA₃ (1 or 5 mg dm⁻³) on growth of *C. murale* plants induced at the phase of the 2nd and the 3rd pair of leaves (Table 2) were similar to those obtained for plants induced at the phase of the 1st pair of leaves (Table 1).

Comparing parameters of growth with percentage of flowering of *C. murale* plants, grown under inductive conditions, high negative correlation (*r* = - 0.80) between effect of glucose and GA₃ concentrations on average values of hypocotyl length and percentage of flowering, was found. Also high positive correlation (*r* = 0.82) was

found between average values of number of leaves and percentage of flowering. In other words, flowering stimulation is associated with inhibition of hypocotyl elongation and acceleration of leaf initiation. This correlation was also mentioned by Pavlová *et al.* (1989b) in *C. murale* and by Seidlová and Sádliková (1983) in *C. rubrum*.

We assume a specific effect of glucose on flowering of *C. murale* plants. GA₃, by showing cumulative stimulatory effect with glucose on flowering of *C. murale* plants, probably affected flower development, rather than

flower initiation. Our results, also, show that in *C. murale* grown *in vitro*, photoperiodic sensitivity depended on the

age of the plants, as it was also pointed *in vivo* by Pavlová *et al.* (1989b).

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