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BRIEF COMMUNICATION

Effect of darkness on growth and flowering of *Chenopodium rubrum* and *C. murale* plants *in vitro*

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Abstract

Chenopodium rubrum, a short-day plant, and *C. murale*, a long-day plant, were grown *in vitro* in continuous darkness. Control *C. rubrum* plants exposed to continuous darkness for 15 d at cotyledonary phase, did not flower, while 80 % of plants flowered on the medium with 5 % glucose and 10 mg dm^{-3} GA_3. Control *C. murale* plants exposed to continuous darkness for 10 d at the age of 4th pair of leaves, did not flower, while GA_3 (1 - 5 mg dm^{-3}) stimulated flowering up to 65 %.

Additional key words: gibberellic acid, glucose, stage of development, long-day plant, short-day plant.

Transition from vegetative to reproductive phase of development is controlled by genetical (autonomous) and ecological (photoperiod and/or temperature) factors. Autonomous control that depends on plant age is the basic control level. Induced or photoperiodic control, alone or in combination with temperature, is the second level of flowering control that stimulates or inhibits genetically determined flowering. The photoperiodic control is loosened with aging in some plants (Bernier *et al.* 1981). Transferring of the plants to darkness cancels photoperiodic control, so the flowering is controlled only by the autonomous mechanism (Chaïlakhyan 1988).

Some long-day (*Beta vulgaris*, *Triticum aestivum*) and short-day (*Pharbitis nil*) plants have been observed to flower in darkness (Fife and Price 1953, Sugino 1957, Inouye *et al.* 1964, Takimoto 1960). According to Bernier *et al.* (1981) sugars in the media are necessary for flowering in darkness. Development and flowering of *Arabidopsis* were also obtained under complete darkness on liquid mineral medium (Rédei *et al.* 1974, Araki and Komeda 1993) or vertical solid agar medium (Roldán *et al.* 1999), supplemented with sucrose.

Gibberellins, exogenously applied, induce or promote flowering in many species being particularly effective in long-day plants as substitute for inductive conditions (Evans 1999). GA_3 stimulated flowering under inductive conditions both in green and photobleached *C. rubrum* (Živanović *et al.* 1995) and in *C. murale* plants (Mitrović *et al.* 2000), but could not be a substitute for inductive photoperiodic regime.

General aim of this study was to provide new informations about flowering in darkness and to compare them with data obtained in green and photobleached *C. rubrum* (Živanović *et al* 1995) and *C. murale* plants (Mitrović *et al.* 2000) exposed to inductive photoperiodic regime.

The experiments were carried out with intact *C. rubrum* plants, ecotype 184, and *C. murale*, ecotype 197, derived from seeds sown *in vitro*. Uniform germination of seeds were attained by temperature and light regimes previously described (Živanović and Ćulafić 1992) for *C. rubrum*. Culture medium sustained of modified Hoagland's mineral solution (Wagner and Leonhard 1985), gelled with 0.62 % agar and supplemented with glucose (2 - 10 %) and/or GA_3

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Abbreviations: GA_3 - gibberellic acid; LD - long day; SD - short day.
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(1 - 10 mg dm^{-3}); pH was adjusted to 5.7 before autoclaving. The temperature in growing room was kept on 23 ± 2 °C. *C. murale* plants were grown on two-phase media (fresh liquid media were added every 10 - 15 d on media solidified with agar) because the long duration of experiments.

C. rubrum seedlings with fully developed cotyledons (5-d-old) were exposed to 15 d of continuous darkness. *C. murale* seedlings with fully developed cotyledons (5-d-old) were grown under non-inductive conditions (photoperiod 8 h) provided by 4 fluorescent tubes in combi-nation with 2 incandescent tubes (4 × 18 W and 2 × 60 W, *Linestra-Osram*, Germany; irradiance 70 μmol m^{-2} s^{-1}) until the 4th pair of leaves was developed, and then transferred to 10 d of continuous darkness or to 10 d of continuous incandescent light, which are inductive for *C. murale* (Mitrović *et al.* 2000), *in vitro* (control) or *in vivo*. Following the darkness treatment the plants were transferred back to non-inductive conditions for another 30 d.

Evaluation of flowering (percentage of flowering plants) and measurement of growth parameters (hypocotyl, epicotyl, 1st internode and number of leaves preceding the first flower) were performed at the end of each experiment (repeated twice). A fully developed terminal flower was taken as a criterion for flowering. The significance of differences between various treatments was evaluated by means of PC program *Statgraphics* (one-way analysis of variance).

Short-day *C. rubrum* plants, exposed to 15 d of continuous darkness, on glucose-free media (control), did not flower which is in agreement with Bernier *et al.* (1981) as sugars in the media are necessary for flowering in darkness. Glucose in all tested concentrations (2 - 10 %) stimulated flowering (Table 1). In darkness 54 % of plants flowered on glucose (2 %). On the same medium under inductive conditions (6 SD + 9 LD) flowered 85 % of plants (Ćulafić 1999). GA_3 stimulated flowering alone or in combination with glucose in the media (Table 1). GA_3 (1 or 5 mg dm^{-3}) stimulated flowering to 5 % on glucose-free medium. Maximal flowering (80 %) was achieved on the medium with 5 % glucose and 10 mg dm^{-3} GA_3. This is the mutual effect of glucose and GA_3, as 50 % of plants flowered on the medium with 5 % glucose. Under inductive conditions, 100 % of flowering was achieved in green *C. rubrum* (Ćulafić 1999) on 5 % glucose and in photobleached plants (Živanović *et al.* 1995) on 5 % glucose and GA_3 (0.1 - 10 mg dm^{-3}).

C. rubrum plants, grown under continuous darkness, were elongated and showed an etiolated appearance. Glucose (3 - 10 %) inhibited hypocotyl elongation

Table 1. The effect of glucose and GA_3 on growth and flowering of *C. rubrum* plants exposed to continuous darkness for 15 d at cotyledonary phase *in vitro* (evaluation of flowering and measurement of growth parameters at the end of experiment). Means ± SE, * - significant against respective control at $P = 5$ %.

Glucose [%]	GA_3 [mg dm^{-3}]	Hypocotyl length [mm]	Epicotyl length [mm]	Number of leaves	Flowering [%]
0	0	23.73 ± 1.61	0.00 ± 0.00	0.00 ± 0.00	0
0	1	25.69 ± 1.17	0.27 ± 0.27	0.18 ± 0.18	5
0	5	18.73 ± 0.84*	0.18 ± 0.13	0.18 ± 0.13	5
0	10	14.00 ± 0.69*	0.00 ± 0.00	0.00 ± 0.00	0
2	0	25.54 ± 1.48	7.58 ± 1.05*	2.08 ± 0.21*	54
2	1	21.95 ± 1.61	4.50 ± 0.54*	1.90 ± 0.23*	60
2	5	16.85 ± 0.98*	4.70 ± 0.38*	2.00 ± 0.00*	70
2	10	14.29 ± 0.51*	3.71 ± 0.25*	2.29 ± 0.16*	43
3	0	21.45 ± 0.48	8.75 ± 0.71*	2.00 ± 0.00*	50
3	1	21.78 ± 0.60	6.61 ± 0.53*	2.17 ± 0.12*	74
3	5	17.05 ± 0.58*	4.86 ± 0.48*	2.86 ± 0.26*	67
3	10	16.07 ± 0.26*	4.48 ± 0.62*	2.89 ± 0.31*	56
5	0	20.74 ± 0.80	10.26 ± 0.90*	2.26 ± 0.14*	50
5	1	20.29 ± 0.81*	9.75 ± 0.62*	2.58 ± 0.19*	79
5	5	15.05 ± 0.79*	6.14 ± 0.66*	2.86 ± 0.22*	71
5	10	14.67 ± 0.46*	5.23 ± 0.35*	3.00 ± 0.19*	80
7	0	17.75 ± 1.05*	8.21 ± 0.86*	2.33 ± 0.16*	29
7	1	18.95 ± 0.68*	9.38 ± 0.77*	2.86 ± 0.26*	67
7	5	13.25 ± 0.76*	4.81 ± 0.48*	3.00 ± 0.26*	75
7	10	14.38 ± 0.40*	4.13 ± 0.32*	3.08 ± 0.21*	58
10	0	15.75 ± 0.86*	4.05 ± 0.81*	1.80 ± 0.29*	25
10	1	16.06 ± 0.47*	8.88 ± 0.73*	2.38 ± 0.20*	38
10	5	12.61 ± 0.54*	4.56 ± 0.49*	3.17 ± 0.23*	39
10	10	10.52 ± 0.25*	3.08 ± 0.27*	2.44 ± 0.20*	28

(Table 1) which is in agreement with results obtained in *C. rubrum* plants either under inductive or non-inductive conditions (Živanović *et al.* 1995). Glucose stimulates development of epicotyls and foliage leaves under continuous darkness, as the plants did not develop epicotyls on the medium without glucose (Table 1). GA_3 (5 or 10 mg dm^{-3}) inhibited *C. rubrum* hypocotyl elongation under continuous darkness (Table 1). GA_3 had no effect on epicotyl elongation and number of leaves (Table 1). Under inductive conditions (Seidlová *et al.* 1990, Živanović *et al.* 1995) GA_3 stimulated 1st internode elongation.

High correlation (r = 0.81) between the effect of glucose and GA_3 concentration on average values of number of leaves and percentage of flowering in darkness was found. Seidlová and Sádliková (1983) also mentioned that the transition to the reproductive phase of development is accompanied by accelerated leaf initiation.

In order to compare induction (6 SD + 9 LD) in *C. rubrum* (Živanović *et al.* 1995), with the exposure to continuous darkness, inductive 6 SD were replaced by 6 d of continuous darkness (unpublished data). On the control medium percentage of flowering (69 %) was lower, compared to induction by 6 SD (81 %) (Živanović *et al.* 1995, Ćulafić 1999). Glucose (2 %) increased flowering to 75 %, similar to inductive conditions (6 SD + 9 LD) (Ćulafić 1999) where 85 % plants flowered, while photobleached, induced (6 SD + 9 LD) *C. rubrum* plants (Živanović *et al.* 1995) did not flower on the same medium.

Flowering of long-day *C. murale* plants *in vitro* was achieved by exposure to 10 d of continuous darkness at the age of 4th pair of leaves (Table 2). On the media supplemented with glucose (5 %) and GA_3 (1 or 5 mg dm^{-3}) 42 or 65 % of plants flowered, respectively. On the same media, 40 or 43 % of *C. murale* plants flowered if they were induced by 10 d of continuous light at the age of 1st pair of leaves (Mitrović *et al.* 2000). *C. murale* plants did not survive on glucose-free medium to the end of experiments, while on glucose-containing (5 %) media (control), there was no flowering (Table 2). Plants induced by 10 d of continuous light at the age of 4th pair of leaves on media supplemented with glucose (5 %) and GA_3 (1 mg dm^{-3}) flowered at 60 % (Table 2). Plants grown *in vivo* under the same photoperiodic conditions flowered to 58 % (Table 2). These results point to increased "capacity" for flowering with aging. Long-day plant *Rudbeckia bicolor* (Chaïlakhyan 1988) also flowered by exposure to darkness at the age of 5 - 7 months *in vivo*.

The results presented are significant both for *Chenopodium rubrum* and *C. murale* plants, as these two species with different photoperiodic demands, have the ability to be induced for flowering in total darkness *in vitro*. The presence of glucose in the media partly compensated the lack of photosynthetic products in darkness. GA_3 stimulated flowering in darkness both in *C. rubrum* and *C. murale* plants. *C. murale* plants loose demands for specific day length with aging (4th pair of leaves), so that transferring to darkness cancels photoperiodic control and flowering is controlled by autonomous mechanism.

Table 2. The effect of glucose and GA_3 on growth and flowering of *C. murale* plants exposed to 10 d of continuous darkness in the phase of 4th pair of leaves *in vitro* (60 - 80 SD + 10 DCD + 30 SD), induced by 10 d of continuous light in the phase of 4th pair of leaves *in vitro* (75 SD + 10 DCL + 30 SD), and induced by 10 d of continuous light in the phase of 4th pair of leaves *in vivo* (50 SD + 10 DCL + 60 SD); DCD - continuous darkness, DCL - continuous light, SD - short day (8 h) (evaluation of flowering and measuring of growth parameters at the end of experiment). Means ± SE, n = 25, * - significant against respective control at P = 5 %.

	Glucose [%]	GA_3 [mg dm^{-3}]	Hypocotyl length [mm]	1st internode length [mm]	Number of leaves	Flowering [%]
60 - 80 SD + 10 DCD + 30 SD	5	0	16.35 ± 0.75	2.86 ± 0.27	9.71 ± 0.66	0
	5	1	17.57 ± 0.99	7.84 ± 0.55*	9.05 ± 0.52	42
	5	5	19.41 ± 0.66*	9.64 ± 0.89*	8.88 ± 0.33	65
75 SD + 10 DCL + 30 SD	5	1	20.00 ± 0.89	10.20 ± 1.02	8.00 ± 0.00	60
50 SD + 10 DCL + 60 SD	0	0	20.17 ± 1.59	7.75 ± 0.55	14.33 ± 0.64	58

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